JOURNAL OF AGRICULTURAL RESEARCH

VOL. 55 WASHINGTON, D.C., OCTOBER 15, 1937 No. 8

DISTRIBUTION AND VARIATION WITH MATURITY OF DISSOLVED SOLIDS, SUCROSE, AND TITRATABLE ACIDITY IN THE SORGO STALK[1]

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EARLIER WORK

Joulie [2] in his earlier work on sorgo as a source of sugar reported the relative weights of the internodes and the ash and sugar content of the juices expressed from them separately. Subsequent investigators ascribed various qualities to the juices expressed from the upper and lower portions of the sorgo stalk, and made widely varying recommendations for topping sorgo for sirup or sugar manufacture.

During the latter half of the nineteenth century, when sorgo was extensively investigated as a commercial source of sugar, there was some confusion regarding the part of the stalk best suited for commercial utilization. Studies made at that time were largely on gross sections of the sorgo stalk, such as thirds and halves, instead of internode divisions. Collier [3] concluded that there was no marked difference in the quantity or quality of the juices from the upper and lower half of the sorgo stalk. He based his conclusions on determinations of specific gravity and total amount of dissolved solids in the juice. Wiley [4] concluded from analyses of thirds of the stalk that at full maturity there was practically no difference in the juice from the upper and lower halves of the sorgo stalk. On the other hand, he [5] stated that studies of Amber cane in Italy seemed to show that the upper internodes and the peripheric strata contained a greater abundance of saccharose than the lower internodes and the central strata.

Martin, Harris, and Jones,[6] in a study of the freezing-point depression and specific conductivity of sorghum tissue fluids, found that when sorgo plants were nearly mature, that is, in the "dough-to-ripe" stage, which is the stage of maturity usually considered best for sirup making, the dissolved solids content of the juice was not the same in the different internodes. It increased from the first to the fourth internode; in the fifth and sixth it was about the same as in the fourth.

More recently one of the writers,[7] in a preliminary study of the quality of sirup produced from various portions of the stalk, corrob-

[1] Received for publication May 19, 1937; issued November 1937.

[2] JOULIE, H. ÉTUDES ET EXPÉRIENCES SUR LE SORGHO À SUCRE CONSIDERÉ AU POINT DE VUE BOTANIQUE, AGRICOLE, CHIMIQUE, PHYSIOLOGIQUE ET INDUSTRIEL. 200 pp., illus. Paris. 1864. (See pp. 101, 144, 145.)

[3] COLLIER, P. REPORT OF THE CHEMIST OF THE AGRICULTURE DEPARTMENT TO COMMISSIONER W. G. LE DUC. Letter of the Commissioner of Agriculture to Honorable J. W. Johnston, Chairman, United States Senate Committee on Sorghum Sugar. 42 pp. 1880. (See p. 26.)

[4] WILEY, H. W. DIFFUSION, ITS APPLICATION TO SUGAR-CANE AND RECORD OF EXPERIMENTS WITH SORGHUM IN 1883. U. S. Dept. Agr., Chem. Div. Bull. 2, 36 pp., illus. 1884. (See p. 24.)

[5] ——— THE NORTHERN SUGAR INDUSTRY. A RECORD OF ITS PROGRESS DURING THE SEASON OF 1883. U. S. Dept. Agr., Chem. Div. Bull. 3, 118 pp., illus. 1884. (See p. 79.)

[6] MARTIN, J. H., HARRIS, J. A., and JONES, I. D. FREEZING-POINT DEPRESSION AND SPECIFIC CONDUCTIVITY OF SORGHUM TISSUE FLUIDS. Jour. Agr. Research 42: 57–69. 1931.

[7] VENTRE, E. K. A STUDY OF THE SORGO STALK WITH REFERENCE TO ITS SIRUP MAKING QUALITIES. Presented at Amer. Chem. Soc. meeting, San Francisco, Aug. 9–13, 1935.

orated, in the main, the conclusions of Martin, Harris, and Jones. In considering, therefore, the contradictory results reported by investigators since the time of Joulie, the need for a more detailed study of the sorgo stalk was apparent. It was thought that such a study might form a basis for selection of that portion of the sorgo stalk most suited for efficient commercial utilization, and possibly throw some additional light on the failure of sorgo in the past as a commercial source of sugar.

PRESENT WORK

PROCEDURE

In cooperation with the Mississippi Agricultural Experiment Station, the four varieties of sorgo (*Holcus sorghum* var. *saccharatus*) most used for sirup production in that section were selected for study. The different varieties, designated by the names locally assigned to them, presented the following varietal characteristics:

Hodo.—A large-barrel, long-stalk, late-maturing variety.

Iceberg.—A small-barrel, short-stalk, early-maturing variety.

Honey.—A small-barrel, medium-height stalk, intermediate-maturing, sprangle-top variety.

Gooseneck.—A medium-barrel, medium-height stalk, intermediate-maturing variety.

The stages of maturity selected for study were determined by the average maturity of the seed heads, described as follows:

Milk stage.—When the seed heads were out of the sheath and well formed, and the contents when pressed out had the consistence of milk.

Dough-to-ripe stage.—When the contents pressed from the seed heads had the consistence of stiff dough, the stage just preceding the solid stage.

Dead-ripe stage.—When the contents of the seed heads were solid, and the glumes were wide open and dry.

When the sorgo had reached the selected stage of maturity, the stalks were cut even with the top surface of the row and stripped of leaves. The seed head and peduncle were removed, and the stalks divided at the nodes. The internodes were numbered consecutively from the peduncle and segregated in batches according to number. Each batch was weighed and ground separately in a horse-driven farm mill. Each was passed through the mill twice in order to extract as much juice as possible with this type of milling equipment.

A whole-stalk sample of each variety at each stage of maturity was used for comparison. An aliquot portion of the stalks was selected and stripped clean, and after the seed heads and peduncle were removed, it was subjected to exactly the same treatment as the various internode batches.

The juice expressed from each set of internodes was collected separately and weighed, and the following determinations were made on the expressed juice:

Dissolved solids or degrees Brix.—Determined by a Brix hydrometer calibrated at 20° C.

Sucrose.—Determined polariscopically after defecation with Horne's dry lead.[8]

Titratable acidity.—A 10-ml portion was titrated with 0.1 normal sodium hydroxide. Phenolphthalein was used as an indicator.

[8] SPENCER, G. L., and MEADE, G. P. A HANDBOOK FOR CANE-SUGAR MANUFACTURERS AND THEIR CHEMISTS. Ed. 7, rewritten and enl. by G. P. Meade, 560 pp., illus. New York and London. 1929. (See p. 225.)

DATA

Tables 1 to 4 give the data obtained for the four varieties of sorgo at different stages of maturity.

Column 2 of each table lists the weight of the stalk in each internode batch.

Column 3 lists the percentage of the total weight contributed by each batch of internodes. The figures are obtained by dividing the weight of each batch of internodes (column 2) by the total weight of stalks ground, or the total of column 2.

Column 4 lists the weight of the juice expressed from each batch of internodes.

TABLE 1.—*Yield and composition of juice from various internodes of Gooseneck variety of sorgo at different stages of maturity*

MILK STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*[1]	*Pct.*	*Lb.*		*Cc*	*Cc*
1	15.0	3.44	6.0	40.00	12.12	0.73	2.37	1.59	0.09	13.11	3.2	2.51
2	23.0	5.27	12.0	52.17	12.96	1.55	5.03	2.97	.36	22.91	2.5	1.84
3	33.0	7.57	19.0	57.57	13.36	2.54	8.24	3.52	.67	26.30	1.5	1.06
4	41.0	9.40	24.0	58.53	13.30	3.19	10.34	3.64	.87	27.36	1.4	1.00
5	44.0	10.10	24.0	54.54	13.56	3.25	10.54	4.34	1.04	32.00	.8	.56
6	47.0	10.78	26.0	55.31	13.45	3.50	11.35	4.69	1.22	34.86	.7	.49
7	50.0	11.47	28.0	56.00	13.22	3.70	12.00	4.97	1.39	37.59	.7	.50
8	50.0	11.47	28.0	56.00	12.62	3.53	11.44	4.21	1.18	33.35	.7	.53
9	49.0	11.24	28.0	57.14	12.50	3.50	11.35	.89	.25	7.12	.7	.54
10	44.0	10.09	24.0	54.54	11.90	2.85	9.24	.60	.14	5.04	.7	.56
11	31.0	7.11	16.0	51.61	12.00	1.92	6.22	.50	.08	4.16	.8	.64
12	9.0	2.06	5.0	55.55	11.59	.58	1.88	.45	.02	3.88	.8	.66
Total or average	436.0	100.00	240.0	55.04	12.85	30.84	100.0	3.05	7.31	23.70	------	.74

DOUGH-TO-RIPE STAGE

1	16.0	3.15	6.0	37.50	15.88	0.95	2.47	7.09	0.45	44.64	3.9	2.31
2	21.0	4.13	9.0	42.85	17.02	1.53	3.97	7.91	.71	46.47	3.0	1.65
3	28.0	5.51	14.5	51.78	17.22	2.49	6.46	9.26	1.34	53.77	2.2	1.19
4	34.0	6.69	19.0	55.88	16.82	3.19	8.28	8.86	1.68	52.67	1.5	.84
5	42.0	8.27	22.0	52.38	16.02	3.52	9.14	7.63	1.67	47.62	1.5	.88
6	50.0	9.84	27.0	54.00	15.12	4.08	10.59	6.43	1.73	42.52	1.4	.87
7	62.0	12.21	34.0	54.84	14.52	4.93	12.80	5.14	1.74	35.40	1.2	.78
8	69.0	13.58	38.0	55.07	14.22	5.40	14.02	4.49	1.70	31.57	1.1	.73
9	69.0	13.58	34.0	49.27	14.38	4.89	12.69	3.88	1.32	26.98	1.1	.72
10	58.0	11.42	29.0	50.00	14.18	4.11	10.67	3.84	1.11	27.08	1.1	.73
11	39.0	7.68	18.0	46.15	13.78	2.48	6.44	3.60	.65	26.12	1.0	.69
12	20.0	3.94	7.0	35.00	13.58	.95	2.47	3.01	.21	22.16	.9	.63
Total or average	508.0	100.00	257.5	50.68	14.95	38.52	100.00	5.55	14.31	37.15	------	.87
Whole stalk	74.0	------	42.0	56.75	14.46	------	------	7.29	------	50.40	1.6	1.04

[1] Of total.

TABLE 1.—*Yield and composition of juice from various internodes of Gooseneck variety of sorgo at different stages of maturity*—Continued

DEAD-RIPE STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*		*Cc*	*Cc*
1	15.0	3.31	7.00	46.66	16.81	1.18	3.09	8.73	0.61	51.93	4.4	2.45
2	20.5	4.51	10.25	50.00	17.91	1.83	4.80	10.23	1.05	57.11	3.8	1.98
3	26.5	5.83	13.50	50.94	18.31	2.47	6.48	11.42	1.54	62.37	2.9	1.47
4	32.5	7.15	18.00	55.38	18.31	3.29	8.63	11.52	2.07	62.92	2.5	1.27
5	38.0	8.36	20.00	52.63	18.11	3.62	9.49	10.33	2.07	57.04	2.2	1.13
6	43.5	9.57	22.00	50.57	17.71	3.89	10.20	9.96	2.19	56.23	2.0	1.05
7	51.5	11.33	25.00	48.54	17.01	4.25	11.13	9.11	2.28	53.55	1.9	1.04
8	58.0	12.76	30.00	51.72	16.61	4.98	13.06	8.40	2.52	50.57	1.8	1.01
9	58.5	12.87	29.00	49.57	16.54	4.79	12.56	6.69	1.94	40.45	1.8	1.02
10	54.0	11.88	24.00	44.44	16.44	3.94	10.33	6.44	1.55	39.17	1.7	.97
11	39.0	8.58	17.00	43.58	16.24	2.76	7.24	6.60	1.12	40.64	1.5	.87
12	17.5	3.85	7.00	40.00	16.14	1.13	2.97	6.41	.45	39.71	1.5	.87
Total or average	454.5	100.00	222.75	49.00	17.11	38.13	100.00	8.70	19.39	50.85	------	1.15
Whole stalk	73.5	------	42.00	57.14	15.87	------	------	8.95	------	56.39	2.1	1.24

TABLE 2.—*Yield and composition of juice from various internodes of Honey variety of sorgo at different stages of maturity*

MILK STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*		*Cc*	*Cc*
1	23.0	3.39	10.0	43.48	12.56	1.25	2.84	2.91	0.29	23.17	3.3	2.50
2	28.0	4.13	14.0	50.00	13.59	1.90	4.31	4.00	.56	29.43	2.1	1.46
3	36.0	5.31	20.0	55.55	14.19	2.84	6.46	4.95	.99	34.88	1.7	1.13
4	48.0	7.09	28.0	58.33	14.19	3.97	9.02	4.73	1.32	33.33	1.0	.67
5	61.0	9.00	35.0	57.37	13.19	4.61	10.48	3.76	1.32	28.50	1.0	.72
6	70.0	10.33	41.0	58.57	12.59	5.16	11.73	3.37	1.38	26.77	.9	.68
7	79.0	11.66	45.0	56.96	12.29	5.53	12.57	3.00	1.35	24.41	.8	.62
8	90.0	13.29	46.0	51.11	11.79	5.42	12.32	2.53	1.16	21.45	.8	.65
9	86.0	12.69	43.0	50.00	11.59	4.98	11.32	.57	.25	4.92	.8	.66
10	79.0	11.66	37.0	46.83	11.59	4.29	9.75	.65	.24	5.60	.8	.66
11	52.0	7.68	24.0	46.15	11.49	2.76	6.27	.67	.16	5.83	.8	.66
12	25.5	3.77	11.0	43.14	11.71	1.29	2.93	.87	.09	7.43	.8	.65
Total or average	677.5	100.00	354.0	52.25	12.43	44.00	100.00	2.57	9.11	20.68	------	.78
Whole stalk	79.0	------	44.0	55.69	12.22	------	------	2.63	------	21.52	1.2	.93

TABLE 2.—*Yield and composition of juice from various internodes of Honey variety of sorgo at different stages of maturity*—Continued

DOUGH-TO-RIPE STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*		*Cc*	*Cc*
1	24.5	3.47	10.75	43.87	15.74	1.69	3.15	7.26	0.78	46.12	3.9	2.33
2	28.0	3.96	12.50	44.64	17.34	2.16	4.03	9.50	1.18	54.78	2.7	1.45
3	34.0	4.81	16.50	48.52	18.04	2.97	5.53	10.52	1.73	58.31	1.6	.82
4	45.5	6.44	23.50	51.64	18.74	4.40	8.20	10.56	2.48	56.35	1.1	.55
5	60.0	8.49	33.00	55.00	17.87	5.89	10.98	9.53	3.14	53.32	1.0	.52
6	70.0	9.91	37.50	53.57	17.07	6.40	11.93	8.57	3.21	50.20	.9	.49
7	82.0	11.61	41.00	50.00	16.47	6.75	12.58	7.43	3.04	45.11	.9	.51
8	89.0	12.60	46.00	51.68	15.87	7.30	13.60	6.26	2.88	39.44	.9	.53
9	90.0	12.74	39.00	43.33	15.24	5.94	11.07	4.85	1.89	31.82	.9	.55
10	90.0	12.74	37.00	41.11	14.84	5.49	10.23	3.93	1.45	26.48	.9	.57
11	68.0	9.62	24.50	36.03	14.76	3.61	6.73	2.70	.66	18.29	.9	.57
12	25.5	3.61	7.25	28.43	14.66	1.06	1.97	2.12	.15	14.46	.9	.58
Total or average	706.5	100.00	328.50	46.49	16.33	53.66	100.00	6.88	22.59	42.13	------	.64
Whole stalk	70.0	------	34.50	49.29	15.64	------	------	6.43	------	41.11	1.5	.90

DEAD-RIPE STAGE

1	22.5	3.11	9.5	42.22	14.50	1.37	2.03	3.56	0.35	24.55	4.3	2.80
2	26.0	3.60	14.0	53.84	16.40	2.69	3.98	5.42	.76	33.04	3.0	1.71
3	43.5	6.02	18.5	42.52	17.30	3.20	4.74	6.81	1.26	39.36	2.1	1.13
4	45.5	6.29	26.5	58.24	18.10	4.79	7.09	8.39	2.22	46.35	1.8	.92
5	58.0	8.02	36.0	62.06	19.08	6.87	10.18	4.83	1.74	25.31	1.8	.87
6	71.0	9.82	40.5	57.04	18.88	7.64	11.32	5.95	2.40	31.51	1.5	.73
7	79.0	10.93	46.0	58.22	18.36	8.44	12.50	5.42	2.49	29.52	1.2	.61
8	91.5	12.65	50.0	54.64	17.98	8.99	13.32	4.03	2.01	22.41	1.2	.62
9	95.0	13.14	47.5	50.00	17.87	8.49	12.57	2.92	1.38	16.34	1.2	.62
10	97.0	13.42	45.0	46.39	17.67	7.95	11.78	1.32	.59	7.47	1.2	.63
11	67.0	9.27	29.0	43.28	18.17	5.23	7.75	1.79	.52	9.85	1.0	.51
12	27.0	3.73	10.0	37.03	18.57	1.85	2.74	.88	.08	4.73	1.0	.50
Total or average	723.0	100.00	372.5	51.52	18.12	67.51	100.00	4.14	15.80	23.40	------	.78
Whole stalk	70.5	------	40.5	57.44	16.26	------	------	6.95	------	42.74	1.4	.80

TABLE 3.—*Yield and composition of juice from various internodes of Iceberg variety of sorgo at different stages of maturity*

DOUGH-TO-RIPE STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*[1]	*Pct.*	*Lb.*		*Cc*	*Cc*
1	21.5	4.86	12.0	65.53	18.95	2.27	4.60	11.98	1.44	63.21	2.7	1.32
2	29.0	6.56	18.0	62.06	19.25	3.46	7.01	12.88	2.31	66.90	2.4	1.15
3	36.0	8.14	24.0	66.66	19.55	4.69	9.50	13.50	3.24	69.05	1.6	.76
4	44.0	9.94	30.0	68.18	19.63	5.89	11.94	13.35	4.00	68.00	1.5	.71
5	49.0	11.07	32.0	65.30	18.95	6.06	12.28	12.13	3.88	64.01	1.4	.68
6	55.0	12.43	35.0	63.63	18.45	6.45	13.07	11.52	4.03	62.43	1.2	.60
7	59.0	13.33	32.0	54.23	17.85	5.71	11.57	10.62	3.39	59.49	1.1	.57
8	59.0	13.33	35.0	59.32	17.55	6.14	12.44	9.77	3.42	55.66	1.0	.53
9	54.0	12.20	30.0	55.55	17.19	5.16	10.46	8.22	2.46	47.81	1.0	.54
10	36.0	8.14	21.0	58.33	16.79	3.52	7.13	7.06	1.48	42.04	1.0	.55
Total or average	442.5	100.0	269.0	60.79	18.34	49.35	100.0	11.02	29.65	60.08	------	.68
Whole stalk	124.0	------	78.0	62.90	18.25	------	------	11.59	------	63.50	1.5	.76

DEAD-RIPE STAGE

1	18.5	4.86	8.25	44.59	21.44	1.77	4.41	14.58	1.20	68.00	5.5	2.36
2	23.5	6.17	11.25	47.87	22.44	2.52	6.28	16.04	1.80	71.42	4.5	1.83
3	29.0	7.61	15.50	53.44	22.84	3.54	8.83	16.68	2.58	73.02	3.3	1.32
4	37.0	9.71	21.00	56.75	22.44	4.71	11.75	17.03	3.57	75.89	2.7	1.10
5	46.0	12.07	23.00	50.00	22.24	5.11	12.74	15.56	3.58	70.00	2.5	1.03
6	48.0	12.60	24.00	50.00	21.84	5.24	13.07	15.03	3.60	68.80	2.5	1.04
7	50.0	13.12	24.00	48.00	21.64	5.19	12.94	13.96	3.35	64.54	2.2	.93
8	53.0	13.91	24.00	45.28	21.44	5.14	12.82	12.91	3.10	60.21	2.0	.85
9	45.0	11.81	20.00	44.44	21.44	4.29	10.70	10.04	2.00	46.82	2.0	.85
10	31.0	8.14	12.00	38.70	21.64	2.59	6.46	9.66	1.16	44.64	1.5	.63
Total or average	381.0	100.00	183.00	48.03	21.91	40.10	100.00	14.17	25.94	64.69	------	1.09
Whole stalk	70.0	------	40.00	57.14	21.84	------	------	15.57	------	71.29	2.4	1.01

[1] Of total.

TABLE 4.—*Yield and composition of juice from various internodes of Hodo variety of sorgo at different stages of maturity*

MILK STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*[1]	*Pct.*	*Lb.*		*Cc*	*Cc*
1	17.5	2.56	7.75	44.28	13.54	1.05	2.39	2.23	0.17	16.47	4.0	2.80
2	23.0	3.36	11.00	47.82	15.14	1.66	3.76	5.40	.59	35.67	2.9	1.80
3	32.0	4.68	15.25	47.65	16.04	2.45	5.56	7.44	1.13	46.38	2.1	1.23
4	36.5	5.33	18.25	50.00	16.04	2.93	6.64	7.27	1.33	45.32	1.3	1.21
5	43.0	6.28	20.00	46.51	15.57	3.11	7.05	6.77	1.35	43.48	1.1	.66
6	50.5	7.38	25.00	49.50	14.87	3.72	8.44	6.09	1.52	40.95	1.0	.63
7	62.5	9.13	30.00	48.00	14.17	4.25	9.64	5.27	1.58	37.19	.9	.60
8	71.0	10.37	34.50	48.59	13.97	4.82	10.93	4.59	1.58	32.85	.7	.47
9	70.5	10.30	32.50	46.10	13.52	4.39	9.96	3.70	1.20	27.36	.7	.49
10	74.75	10.92	31.75	41.91	13.22	4.19	9.50	3.12	.99	23.60	.7	.50
11	82.50	12.05	34.50	41.81	12.92	4.45	10.09	3.07	1.06	23.76	.7	.51
12	65.75	9.61	30.00	45.62	12.92	3.87	8.78	2.91	.87	22.52	.7	.51
13	34.50	5.04	16.25	47.10	12.97	2.11	4.79	1.98	.32	15.26	.7	.51
14	20.50	2.99	8.50	41.46	12.87	1.09	2.47	1.74	.15	13.52	.7	.51
Total or average	684.5	100.00	315.25	46.05	13.98	44.09	100.00	4.39	13.84	31.39	------	.72
Whole stalk	70	------	38.50	55.00	13.82	------	------	4.73	------	34.22	1.0	.68

DOUGH-TO-RIPE STAGE

1	13.00	2.38	6.0	46.15	19.08	1.14	2.42	11.21	0.67	58.75	3.0	1.46
2	17.50	3.21	10.0	57.14	19.28	1.93	4.09	11.78	1.18	61.09	2.3	1.10
3	21.50	3.94	12.5	58.13	19.28	2.41	5.11	12.02	1.50	62.34	2.0	.96
4	25.00	4.58	14.5	58.00	18.98	2.75	5.83	11.69	1.69	61.59	1.4	.68
5	30.00	5.49	15.5	51.66	18.63	2.88	6.10	10.41	1.61	55.87	1.3	.64
6	35.00	6.41	19.5	55.71	18.23	3.55	7.52	9.85	1.92	54.03	1.1	.56
7	41.00	7.51	22.5	54.87	17.63	3.96	8.39	8.75	1.97	49.63	1.0	.53
8	46.00	8.42	24.5	53.26	17.33	4.24	8.98	8.06	1.97	46.50	1.0	.53
9	53.00	9.71	25.5	48.11	16.62	4.24	8.98	6.59	1.68	39.65	.9	.50
10	60.50	11.08	30.0	49.58	16.32	4.89	10.36	5.97	1.79	36.58	.9	.51
11	61.50	11.26	27.5	44.71	16.18	4.45	9.43	5.48	1.50	33.86	.9	.52
12	62.50	11.45	28.5	45.60	16.18	4.61	9.77	5.38	1.53	33.25	.9	.52
13	47.00	8.61	21.5	45.74	16.60	3.57	7.57	4.55	.98	27.40	.9	.50
14	32.50	5.95	15.5	47.69	16.60	2.57	5.45	4.49	.69	27.04	.9	.50
Total or average	546.00	100.00	273.50	50.09	17.25	47.19	100.00	7.56	20.68	43.82	------	.60
Whole stalk	75.00	------	44.0	58.66	17.10	------	------	9.69	------	56.66	1.5	.82

DEAD-RIPE STAGE

1	12.0	1.89	6.00	50.00	18.40	1.10	1.92	12.01	0.72	65.27	3.2	1.64
2	19.0	2.99	10.00	52.63	18.45	1.84	3.22	12.39	1.23	67.15	2.4	1.21
3	24.0	3.77	13.00	54.16	18.50	2.40	4.20	12.44	1.61	67.24	2.0	1.00
4	27.5	4.33	14.75	53.63	18.50	2.73	4.77	12.44	1.83	67.24	1.7	.85
5	31.5	4.96	16.50	52.38	18.44	3.04	5.32	12.18	2.01	66.05	1.1	.55
6	31.5	4.96	18.00	57.14	18.04	3.24	5.67	11.83	2.13	65.57	1.0	.51
7	46.0	7.24	23.00	50.00	18.04	4.15	7.26	10.96	2.52	60.75	.8	.41
8	53.0	8.34	27.00	50.94	17.27	4.66	8.15	10.24	2.76	59.29	.6	.33
9	59.0	9.28	30.50	51.69	16.77	5.11	8.94	9.13	2.78	54.44	.6	.33
10	67.5	10.62	34.50	51.11	16.65	5.74	10.04	8.78	3.03	52.73	.7	.39
11	77.0	12.12	40.50	52.59	16.60	6.72	11.75	8.67	3.51	52.22	.7	.39
12	74.0	11.64	39.00	52.70	17.00	6.63	11.60	9.16	3.57	53.88	.7	.38
13	67.0	10.54	34.50	51.49	17.21	5.94	10.39	9.72	3.35	56.47	.7	.38
14	46.5	7.32	22.50	48.38	17.21	3.87	6.77	9.69	2.18	56.30	.8	.43
Total or average	635.5	100.00	329.75	51.89	17.33	57.17	100.00	10.07	33.23	58.10	------	.49
Whole stalk	75.0	------	45.00	60.00	16.55	------	------	9.91	------	59.89	.9	.51

Column 5 gives the ratio, expressed as percentage, of the weight of juice expressed from each batch of internodes to the weight of stalks ground in each batch. The footing of this column is the ratio, expressed as percentage, of the total of column 4 to the total of column 2.

Column 6 lists the percentage of dissolved solids in the juice expressed from each batch of internodes. The footing of this column is the average dissolved solids determined by dividing the total of column 7 by the total of column 4.

Column 7 lists the weight, in pounds, of dissolved solids expressed in the juice from each batch of internodes. This is the product of the degrees Brix in column 6 and the corresponding weight of juice in column 4.

Column 8 lists the percentage of the dissolved solids contributed by each batch of internodes to the total dissolved solids expressed from the stalk. This is arithmetically the ratio, expressed in percentage, of the respective components of column 7 to the total of column 7.

Column 9 lists the percentage of sucrose in the juice expressed from each batch of internodes. The footing of this column is the average obtained by dividing the total of column 10 by the total of column 4.

Column 10 lists the pounds of sucrose in the juice expressed from each batch of internodes. The figures are the product of the percentage of sucrose in column 9 and the corresponding weight of juice in column 4.

Column 11 lists the coefficient of apparent purity,[9] a ratio used in commercial sugar manufacture to estimate sucrose available for recovery by crystallization. The values are obtained by dividing the percentage of sucrose in the juice by the degrees Brix and then multiplying by 100. The footing of this column is the average obtained by dividing the footing of column 9 by the footing of column 6 and multiplying by 100.

Column 12 lists the titratable acidity of the juices. This is expressed in the milliliters of 0.1 normal alkali required to neutralize 10 ml of the juice.

Column 13 lists the acidity in milliliters of 0.1 normal alkali calculated on the basis of 1 g of Brix solids. The figures are obtained by multiplying the volume of juice titrated (10 ml) by the specific gravity [10] at the observed degrees Brix, multiplying the product by the observed degrees Brix, and dividing the result into the titer. The footing of this column is the weighted average obtained by multiplying the figures of column 13 by the corresponding figures in column 7, totaling, and dividing by the total of column 7 expressed in like units.

DISCUSSION

Column 3 of table 3 shows that in the short-stalk, early-maturing variety Iceberg, very nearly three-fourths of the weight of the stalk occurred in internodes 4 to 9, inclusive. Internodes 1, 2, 3, and 10 constituted only about one-fourth of the weight of the stalk. With maturity, there was practically no shift in distribution of weight among the internodes.

In the medium-length, intermediate-maturing varieties Gooseneck and Honey (tables 1 and 2) the bulk of the weight of the stalk, about

[9] SPENCER, G. L., and MEADE, G. P. See pp. 357–358 of reference cited in footnote 8.
[10] SPENCER, G. L., and MEADE, G. P. See pp. 459–468 of reference cited in footnote 8.

two-thirds, occurred in internodes 5 to 10, inclusive, at all three stages of maturity.

In the large-barrel, long-stalk, late-maturing variety Hodo (table 4) about three-fifths of the total weight of the stalk was in internodes 7 to 12, inclusive. There was practically no shift in weight with maturity.

Extraction of juice (column 5) was highest in the upper half of the stalk in all the varieties and at the three stages of maturity. The total juice extracted from the internodes was less than that obtained from the whole stalk. That this was partly due to evaporation of moisture from the relatively short internodes before grinding is shown by the higher average Brix of the juice from the internodes as compared with the Brix of the juice from the whole stalk.

The percentage of dissolved solids (degrees Brix, column 6) increased progressively with maturity in all the varieties with the exception of the dead-ripe stage of the Hodo variety and the upper internodes of the dead-ripe stage of the Honey variety. The Honey variety has a sprangle top, and as soon as the seed head matures and opens, the seeds fall to the ground, branching occurs, and the upper portion of the stalk reverts to the immature stage. The failure of the Hodo variety to show the usual increase in dissolved solids from the dough-to-ripe to dead-ripe stage of maturity was due to intervening rain. This was demonstrated by the fact that although there was little change in the average percentage of dissolved solids of the juice in either the whole stalk or the internode, there was an increase in the percentage of juice expressed.

In the main, the percentage of dissolved solids in the juice increased progressively from the first internode to a maximum in the third or fourth, and decreased progressively to the last internode, except where normal growth had been interfered with by branching or rain.

The percentage of solids in the juice from each internode (based on the total expressed solids, column 8) is a measure of the amount of sirup contributed by the juice of each internode to the total sirup obtained from the stalk. This makes it possible to estimate the loss of sirup incurred by discarding a portion of the stalk.

With the exception of the dead-ripe stage of the Honey variety, the percentage of sucrose determined (column 9) and its ratio to dissolved solids, or coefficient of apparent purity (column 11), increased with maturity. Both reached a maximum in about the same internode as the dissolved solids, but in the lower internodes they decreased much more rapidly than the dissolved solids.

Titratable acidity per gram of dissolved solids in the juice (column 13) is a measure of the acidity contributed by the juice to the sirup manufactured from it. One variety, Hodo (table 4), decreased in average acidity with maturity. The Honey variety (table 2) remained very nearly constant, whereas the Gooseneck and Iceberg varieties (tables 1 and 3) increased with maturity. These variations seem to be varietal characteristics.

The titratable acidity per gram of solids for all varieties and stages of maturity studied was highest in the first internode and decreased rapidly in the internodes constituting the upper third of the stalk. Throughout the remainder of the stalk it remained nearly constant at a value generally less than the weighted whole-stalk average and control.

SUMMARY AND CONCLUSIONS

In four varieties of sorgo at different stages of maturity, two-thirds to three-fourths of the weight of the stalk was derived from internodes in the midportion of the stalk.

With the exception of the sprangle-top variety, Honey, which drops its seed and branches after ripening, with partial reversion in the upper internodes to the immature stage of maturity, and the Hodo variety, the juice of which was diluted by rain between the dough-to-ripe and dead-ripe stages of maturity, the following general trends were noted for the four varieties at different stages of maturity:

The percentage of dissolved solids in the juice increases with maturity, and in the main progressively increases from the first to a maximum in the third or fourth internode, with a small progressive decrease in the lower internodes. There is a close correlation between the percentage of dissolved solids and the percentage of weight contributed by each internode.

The percentage of sucrose in the juice increases from the first to a maximum in the third or fourth internode, and progressively and markedly decreases in the lower internodes. With maturity, the total percentage of sucrose in the expressed juice increases, and in the same relative proportion in the different internodes.

The coefficient of apparent purity of the juice parallels closely the percentage of sucrose determined by polarization. It increases totally with maturity.

If sorgo juices are to be used for the crystallization of sucrose, previously recommended topping practices are incorrect. The reverse procedure should be used, that is, the bottom internodes should be discarded, as they have relatively a much lower coefficient of purity. In some cases several internodes at the bottom of the stalk are much below the practical crystallization limits for sucrose.

In the manufacture of sirup from the sorgo stalk, however, topping, or discarding the three or four upper internodes, reduces the tendency of the sirup to crystallize sucrose and also produces sirup with a minimum of acidity or sharp "tang."

FOOT ROT OF CHINA-ASTER, ANNUAL STOCK, AND TRANSVAAL DAISY CAUSED BY PHYTOPHTHORA CRYPTOGEA[1]

By C. M. TOMPKINS, *assistant plant pathologist, California Agricultural Experiment Station*, and C. M. TUCKER, *plant pathologist, Missouri Agricultural Experiment Station*[2]

INTRODUCTION

In August 1934, a destructive foot rot of China-aster (*Callistephus chinensis* Nees) was observed in Golden Gate Park, San Francisco, Calif. Most of the plants which had reached the flowering stage were either dead or dying. The disease, caused by *Phytophthora cryptogea* Pethybr. and Laff., is of general occurrence on the northern end of the San Francisco peninsula. More recently, annual stock or gilliflower (*Matthiola incana* R. Br. var. *annua* Voss), also cultivated extensively in this section, and Transvaal daisy (*Gerbera jamesonii* Hook. var. *transvaalensis* Hort.), grown at Hayward and Capitola, Calif., were found to be similarly affected. The studies reported in this paper deal principally with the disease on China-aster.

REVIEW OF LITERATURE

A disease of China-aster, known as blackleg or foot rot, was reported from England in 1915 by Robinson (*20*),[3] who attributed the cause to an unidentified species of *Phytophthora*.

In 1919, Pethybridge and Lafferty (*16*) described a foot-rot disease of young tomato plants, prevalent in a nursery near Dublin, Ireland. Diseased China-asters with comparable symptoms were found in the same locality. A new species of *Phytophthora*, described as *P. cryptogea*, was cultured from diseased tomato and China-aster plants. The pathogenicity of the two isolates was established by wound inoculations into their respective hosts, while cross inoculations were likewise successful.

Brittlebank and Fish (*6*) reported infection of mature China-aster plants by *Phytophthora cryptogea* in 1927.

The disease was serious on China-asters in England in 1925 and 1926, according to Pethybridge (*15*).

Bewley (*4*) reported the disease on China-asters in England in 1927, and damping-off in 1930.

Pethybridge, Moore, and Smith (*17*) recorded the frequent occurrence of a foot rot of China-aster in 1929 and 1931 in England, and Pethybridge[4] reported its presence in 1933 and 1934.

The first record of the disease on mature China-aster plants in the United States was provided by Tompkins, Tucker, and Clarke (*22*).

[1] Received for publication Apr. 28, 1937; issued November 1937. Joint contribution from the Division of Plant Pathology, California Agricultural Experiment Station, and the Department of Botany, Missouri Agricultural Experiment Station.

[2] Grateful appreciation is expressed to Prof. Ralph E. Smith and Dr. M. W. Gardner, of the California station, for helpful suggestions and assistance; and to Prof. B. A. Madson and W. W. Mackie, Division of Agronomy of the California station, for generously supplying greenhouse facilities for the inoculation tests.

[3] Reference is made by number (italic) to Literature Cited, p. 572.

[4] Letter from George H. Pethybridge dated Aug. 13, 1935.

According to Martin,[5] blackleg of China-asters was found in the District of Columbia in 1926 by Charles Drechsler, who isolated an unidentified species of *Phytophthora* from diseased specimens.

Pethybridge (*15*) and Ware (*23*) have briefly recorded the occurrence of the disease on annual stock or gilliflower (*Matthiola incana* var. *annua*).

Infection of Transvaal daisy (*Gerbera jamesonii* var. *transvaalensis*) has not previously been reported in the literature.

According to published reports, the natural host range of *Phytophthora cryptogea* includes, in addition to China-aster and annual stock, plants of 19 genera in 13 families: Araceae: calla (*Zantedeschia aethiopica* Spreng.) (*11, 17*); Liliaceae: tulip (*Tulipa* sp.) (*1, 10*); Iridaceae: gladiolus (*Gladiolus* sp.) (*1*); Papaveraceae: Iceland poppy (*Papaver nudicaule* L.) (*6*); Cruciferae: cauliflower (*Brassica oleracea* L. var. *botrytis* L.) (*17*), wallflower (*Cheiranthus cheiri* L.) (*6, 16*); Leguminosae: white lupine (*Lupinus albus* L.) (*6*); Umbelliferae: celery seedlings (*Apium graveolens* L.) (*9*); Ericaceae: *Rhododendron carolinianum* Rehd., *R. catawbiense* Michx., *R. maximum* L. (*25*), and *Pieris floribunda* (Pursh) Benth. and Hook.[6]; Primulaceae: primrose (*Primula* sp.) (*6*); Solanaceae: tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey (*2, 6, 8, 16, 17, 21, 24*) and petunia seedlings (*Petunia hybrida* Hort.) (*16*); Scrophulariaceae: snapdragon (*Antirrhinum majus* L.) (*6*); Cucurbitaceae: cucumber (*Cucumis sativus* L.) (*5*); and Compositae: zinnia (*Zinnia elegans* Jacq.) (*12*), dahlia (*Dahlia* sp.) (*17*), African marigold (*Tagetes erecta* L.) (*18*), and cineraria (*Senecio cruentus* DC.) (*3, 6, 16*).[7]

FACTORS FAVORING INFECTION

The principal environmental factors which favor inception and spread of the disease are excessive irrigation, inadequate drainage, and cool weather. The disease is not confined to low, flat areas but may occur on sloping ground if the soil is kept extremely wet. Complete failure of the crop may be expected when soils become waterlogged.

Observations have shown that when plants are grown on well-drained sites and supplied with only enough moisture to satisfy growth requirements, the incidence of disease is greatly reduced.

SYMPTOMS OF THE DISEASE

ON CHINA-ASTER

Damping-off of China-asters, due to *Phytophthora cryptogea*, has not been observed under natural conditions in California, although it has been reported from England (*4, 20*).

Generally the disease does not occur until some weeks after the greenhouse-grown seedlings have been transplanted to beds out of

[5] MARTIN, G. H. DISEASES OF ORNAMENTALS. CALLISTEPHUS CHINENSIS, CHINA-ASTER. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 55: 352-363. 1927. [Mimeographed.]

[6] HUMPHREY, H. B., and WOOD, J. I. DISEASES OF PLANTS IN THE UNITED STATES IN 1933. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 86, 107 pp. 1934. [Mimeographed.]

[7] After this paper was accepted for publication, attention of the writers was called to a recent report on the natural infection of Marguerite (*Chrysanthemum frutescens* L.) by *Phytophthora cryptogea*. [OYLER, E. A DISEASE OF THE MARGUERITE. Nursery and Market Gard. Indus. Devlpmt. Soc., Chestunt, Expt. and Research Sta. Ann. Rept. (1936) 22:59. 1937.] Accordingly, this host should be added to the list given above.

doors. Although a few plants die early in the season, the heaviest loss occurs immediately preceding and during the flowering period (fig. 1). The disease appears suddenly, and infected plants usually die promptly.

Above ground, the chief diagnostic symptoms of the disease consist of rapid, permanent wilting of the leaves; a blackish-brown discoloration of the lower part of the stem; and lodging of the plant on the ground, occasioned by rotting, shriveling, and collapse of the stem at or near the soil line.

The fungus enters the plant through the tap or lateral roots, ultimately spreading throughout the entire root system and upward into

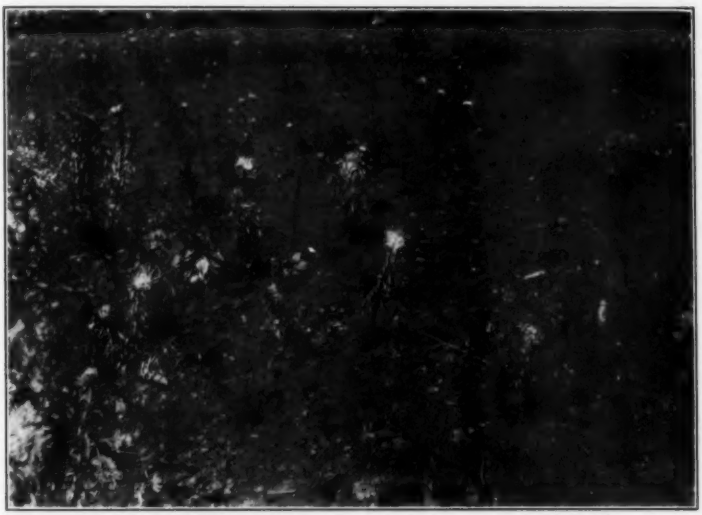

FIGURE 1.—Phytophthora foot rot of China-asters in Golden Gate Park, San Francisco, August 27, 1935. Infection causes rapid wilting, collapse, and sudden death of the plants.

the lower part of the stem (fig.2). The invaded roots and stems show a soft, water-soaked, blackish-brown, odorless type of decay which at first is localized in the cortical parenchyma but later involves all tissues. Sometimes infected plants develop adventitious roots at the upper edge of the diseased part of the root. Diseased plants are easily pulled from the soil, but invariably the cortex of the taproot and lateral roots sloughs off and remains behind.

ON ANNUAL STOCK

The fungus may attack annual stock plants in all stages of growth, but heaviest infection occurs prior to or during the flowering period. The symptoms consist of sudden wilting and drooping of all but the youngest tuft of leaves, infection and rotting of the roots and lower part of the stem, with ultimate breakage of the stem at or near the soil level. The invaded tissues are blackish brown in color, water-soaked, soft, and odorless. Infected plants are easily pulled from the soil.

ON TRANSVAAL DAISY

On Transvaal daisy, symptoms consist of sudden wilting of the leaves accompanied by a conspicuous color change from the normal green to the violet carmine of Ridgway (*19*). Plants of all ages are

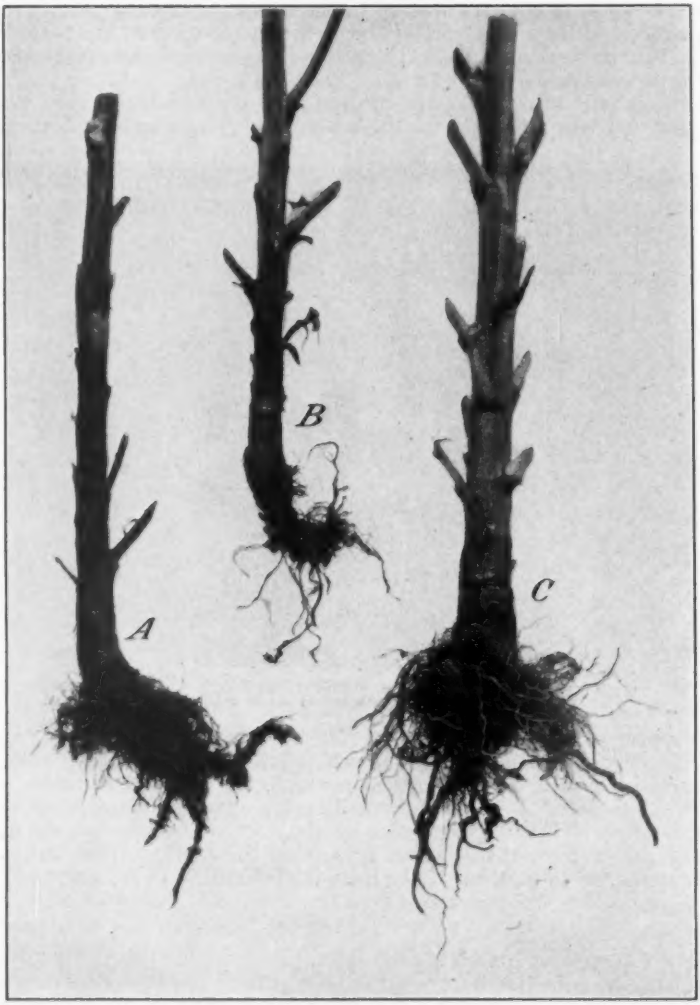

FIGURE 2.—Phytophthora foot rot of China-aster (natural infection): *A* and *B*, Decay of the root system and typical lesions on the lower part of the stems; *C*, healthy root system and stem

susceptible, but the principal damage occurs during the flowering period. After invading the crown tissues at or just below the soil level, the fungus travels downward into the roots. Diseased crown and root tissues are soft, water-soaked, blackish brown in color, and

odorless. When infected plants are dug, the cortex of the roots usually sloughs off, exposing the discolored central cylinder or stele. If an attempt is made to pull the plant before digging, breakage occurs at the crown and most of the roots remain in the soil.

THE CAUSAL FUNGUS, PHYTOPHTHORA CRYPTOGEA

Phytophthora cryptogea was readily isolated from the roots and stems of recently infected China-aster, annual stock, and Transvaal daisy plants by placing small tissue fragments from the internal, advancing margin of decay on malt-extract agar (*13*). After 48 hours, pure cultures were established on agar slants by transferring hyphal tips from Petri-dish colonies. The fungus was cultured at intervals from approximately 100 diseased China-aster and 50 diseased annual stock plants collected in different localities on the San Francisco peninsula. It was also isolated from numerous diseased Transvaal daisy plants. From a group of diseased Transvaal daisy plants collected at Burlingame, Calif., pure cultures of *P. drechsleri* Tucker were consistently obtained.

Freehand cross and longitudinal sections of naturally and artificially infected roots and stems of China-aster, annual stock, and Transvaal daisy were stained with fast green and Magdala red. The fungus is intercellular. Pethybridge and Lafferty (*16*) found the mycelium to be intercellular and intracellular in the parenchymatous tissues of tomato plants. The hyphae, upon invading healthy tissues, were confined to the intercellular spaces, but in very young seedlings and in older diseased plants were observed in the wood vessels.

Isolates of *Phytophthora cryptogea* from China-aster, annual stock and Transvaal daisy were indistinguishable in culture. On potato-dextrose agar there was scant aerial growth of white mycelium with the development of occasional clusters of vesicular swellings in the mycelium; on Difco corn-meal agar the mycelial growth was almost entirely submerged; on oatmeal agar the development of aerial mycelium was fairly profuse but no spores appeared. Sporangia and oogonia failed to develop on any of the solid culture media used.

The vesicles that appeared in potato-dextrose agar cultures were usually less than 27 microns in diameter. They were spherical to irregular in shape, thin-walled, with hyaline contents that resembled those of the hyphae, from which they were not separated by walls as are the chlamydospores produced by *Phytophthora parasitica* and other species. The vesicles of *P. cryptogea* differed from those of *P. cinnamomi* in their smaller size, comparative rarity in culture, and in their smaller degree of differentiation from the hyphae.

Sporangia developed fairly abundantly on hyphae transferred from week-old cultures on oatmeal agar or in pea broth to Petri's solution, distilled water, or nonsterile soil leachate, as suggested by Mehrlich (*14*); in the latter the sporangia developed very promptly, appearing after 48 hours. They were obpyriform to ovate or elongated, the latter often slightly constricted near the middle, nonpapillate, with a small distinct refringent thickening at the apical end, 22.8 to 44.8 by 11.6 to 21.2 microns, averaging 32.2 by 17.2 microns. Sporangia produced after 6 days in Petri's solution were similar in shape and size to those produced in the nonsterile soil leachate. The

sporangia germinated by the growth of a single slender hypha from the refringent apical region, or by the development of zoospores which were fully differentiated within the sporangium and escaped in the manner typical of the genus. The sporangiophores resumed growth through evacuated sporangia in the fashion usual in *Phytophthora cryptogea* and certain other species with nonpapillate sporangia.

Oogonia, oospores, and antheridia were not observed in the cultures examined, and late and scanty development of sexual spores in culture is probably characteristic of the species.

Nine isolates were grown on Difco corn-meal agar plates at various temperatures. After 96 hours the average diameters of the mycelial growths were as follows: At 3° to 4.5° C., 0; at 11°, 15 mm; at 15°, 35 mm; at 20°, 50 mm; at 25°, 59 mm; at 30°, 48 mm; at 35°, 0. At 35° the inocula usually failed to survive the exposure of 96 hours.

The California isolates of *Phytophthora cryptogea* were compared in parallel cultures with an isolate of the species sent to the Centraalbureau voor Schimmelcultures by Pethybridge. Their similarity in cultural characters, morphology, and temperature relations seem to justify the identification of the California isolates with the aster parasite reported from Europe and Australia, even in the absence of observations on the sexual stages.

PATHOGENICITY TESTS

Inoculum was prepared by growing the different isolates of the fungus on sterilized, moistened cracked wheat. It was added in uniform quantity to autoclaved soil in 6-inch pots, each containing a young plant (6 to 10 leaves), in a manner designed to avoid wounding the roots. Sterilized wheat was used for the noninoculated controls. The plants, grown in a greenhouse at temperatures ranging from 18° to 22° C., were watered heavily each day to provide favorable conditions for infection.

ON CHINA-ASTER

The pathogenicity of the China-aster isolate was proved on the variety Late Branching Mary Semple. On December 28, 1934, 25 plants were inoculated. By January 9, 1935, all plants were infected, the incubation period ranging from 5 to 12 days. Symptoms shown by diseased plants were identical with those resulting from natural infection. Typical wilting of the leaves (fig. 3) and collapse of the stem with lodging of the plant occurred. When diseased plants were pulled from the soil, the soft, watery, blackish-brown cortical tissues were readily sloughed off from the central cylinder of the taproot. Most of the lateral roots were completely rotted. The five noninoculated controls continued healthy. All infected roots yielded the fungus when plated.

On January 25, 25 China-aster plants of the same variety were inoculated with the reisolated fungus. All plants were infected by February 5, the incubation period ranging from 7 to 11 days. The five noninoculated controls were healthy.

Damping-off of China-aster seedlings (varieties Apricot and Fordhook Favorite) occurred when seeds were planted in previously infested soil or in autoclaved soil to which the fungus was later added.

ON ANNUAL STOCK

Healthy annual stock plants (variety Fiery Blood Red) were readily infected in 24 days by artificial inoculation with the isolate from this host (fig. 4). The fungus was reisolated and its pathogenicity again demonstrated by inoculation. All control plants remained healthy.

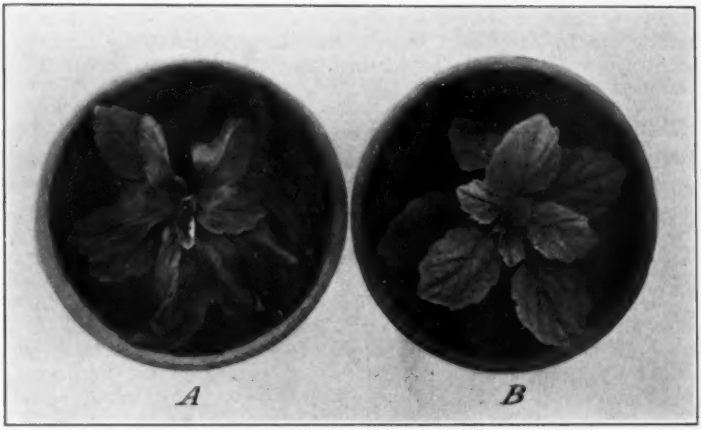

FIGURE 3.—Phytophthora foot rot of China-aster: *A*, Artificial infection of the variety Late Branching Mary Semple after 7 days, showing rapid wilting; *B*, healthy control.

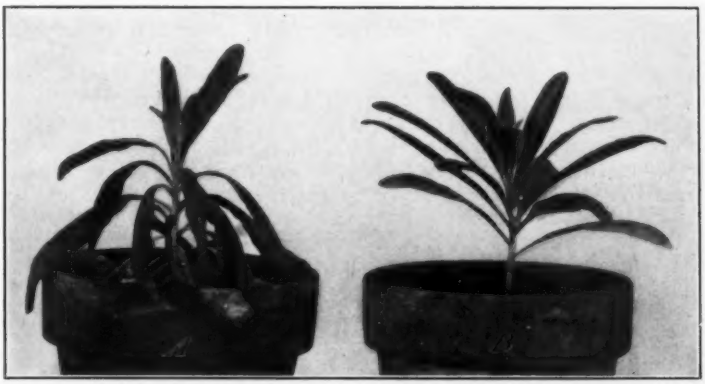

FIGURE 4.—Phytophthora foot rot of annual stock: *A*, Rapid wilting of the outer whorls of older leaves 20 days after inoculation with the isolate from this host, a tuft of young inner leaves remaining turgid; *B*, healthy control. Variety Fiery Blood Red.

ON TRANSVAAL DAISY

Two isolates of *Phytophthora cryptogea* from Transvaal daisy grown in widely separated localities proved pathogenic when inoculated into young, healthy Transvaal daisy plants. Typical symptoms of the disease were produced in 20 to 69 days. The pathogenicity of the two reisolates was again demonstrated by inoculation.

A culture of *Phytophthora drechsleri*, previously referred to, also caused infection of healthy Transvaal daisy plants after inoculation. Infected plants showed symptoms which were identical with those resulting from invasion by *P. cryptogea*, and the incubation period was approximately of the same duration. The desirability of laboratory cultural examinations of diseased specimens is apparent.

It is concluded that the several isolates of the fungus are highly pathogenic to the hosts from which they originated. That direct penetration of unwounded healthy roots and stems occurred under conditions of these tests is indicated.

SUSCEPTIBILITY OF CHINA-ASTER VARIETIES TO INFECTION

Numerous commercial varieties of China-aster were tested for susceptibility to infection by *Phytophthora cryptogea*. For convenience, these varieties were segregated into arbitrary classes:[8] Crego, 36; Dwarf Bouquet, 1; Early Beauty, 24; Giants of California, 14; King, 14; Late Beauty, 4; Late Branching, 39; Peony Flowered, 11; Pompon, 17; Queen of the Market, 20; Royal, 9; Single or Sinensis, 11; Sunshine, 4; Victoria, 10; and unclassified, 13. Of the 227 varieties, 75 were listed as resistant to fusarium wilt by the seedmen from whom they were obtained. Wilt-resistant varieties were not available in the Dwarf Bouquet, Giants of California, Late Beauty, Pompon, and Sunshine classes. In addition, one foreign variety belonging to the King class and of no commercial value because of its dwarf habit, was tested.

Approximately 20 plants of each variety were inoculated according to the method previously described. This involved in the aggregate about 5,000 plants. All varieties proved to be highly susceptible, with no survival of individuals in any particular variety. Under greenhouse conditions, the fusarium wilt-resistant varieties were as readily attacked by the fungus as those listed as wilt susceptible. The incubation period for most varieties ranged from about 10 to 45 days. When considered collectively, the minimum incubation period for all tested varieties was 6 days and the maximum 93 days. Although some infected plants reached the flowering stage before they died, most of them succumbed prior to flower formation.

In the autumn of 1935, seed was obtained from a few apparently resistant China-aster plants grown in Golden Gate Park. Progeny of all selections proved susceptible upon inoculation.

EXPERIMENTAL HOST RANGE

Artificial infection of certain plants by *Phytophthora cryptogea* has been recorded by several investigators, a brief resume of which is given. Pethybridge and Lafferty (*16*) reported infection with an isolate of this fungus from tomato, after wounding, of beech seedlings (*Fagus sylvatica* L.), mangels (*Beta vulgaris* L.), swedes (*B. campestris* L. var. *napobrassica* DC.), white turnips (*B. rapa* L.), apples (*Malus sylvestris* Mill.), *Gilia tricolor* Benth., potato tubers (*Solanum tuberosum* L.), and green and ripe tomato fruits (*Lycopersicum esculentum* var. *vulgare*). Later, Cairns and Muskett (*7*) corroborated in part the earlier work of Pethybridge and Lafferty (*16*) by artificially producing pink rot of potato tubers (*S. tuberosum*).

[8] This classification was kindly suggested by Harry B. Joy of Salinas, Calif.

The China-aster isolate of *Phytophthora cryptogea* has a comparatively limited experimental host range. Young plants (6 to 10 leaves) of wallflower (*Cheiranthus cheiri*), annual stock (*Matthiola incana* var. *annua*), Transvaal daisy (*Gerbera jamesonii* var. *transvaalensis*), Michaelmas daisy (*Aster alpinus* L., *A. oreophilus* Franch., and *A. subcoeruleus* S. Moore var. *diplostephioides* Hort.), and hybrid cineraria (*Senecio cruentus*) were artificially infected. Damping-off of cucumber seedlings (*Cucumis sativus*) occurred when seeds were planted in infested soil.

Fruits of eggplant (*Solanum melongena* L. var. *esculentum* Nees), ripe tomato (*Lycopersicum esculentum* var. *vulgare*) var. Stone, green bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.), pumpkin (*Cucurbita pepo* L. var. *condensa* Bailey) var. Early White Bush Scallop, watermelon (*Citrullus vulgaris* Schrad.), and cucumber (*Cucumis sativus*) were infected without wounding. Lesions on all fruits had a water-soaked appearance except those on eggplant, which were warm sepia in color.

The following species reacted negatively to artificial inoculation: Bulbous plants, including Chinese sacred-lily (*Narcissus tazetta* L. var. *orientalis* Hort.), tulip (*Tulipa gesneriana* L.), hyacinth (*Hyacinthus orientalis* L.), hybrid gladiolus (*Gladiolus* sp.), freesia (*Freesia hybrida* Hort.), and poppy-flowered anemone (*Anemone coronaria* L.); fruits of apple (*Malus sylvestris* Mill.), var. Yellow Newtown, green tomato (*Lycopersicon esculentum* var. *vulgare*) var. Stone, squash (*Cucurbita maxima* Duchesne) var. Banana, pumpkin (*Cucurbita pepo* var. *condensa*) var. Pie, Yellow Crookneck, and Zucchine, Persian melon (*Cucumis melo* L. var. *reticulatus* Naud.), and Honey Dew melon (*C. melo* var. *inodorus* Naud.); roots of garden beet (*Beta vulgaris*), rutabaga (*B. campestris* var. *napobrassica*), turnip (*B. rapa*) var. Purple Top White Globe, carrot (*Daucus carota* L.), and parsnip (*Pastinaca sativa* L.); tubers of potato (*Solanum tuberosum*) var. Russet Burbank; and young plants of Iceland poppy (*Papaver nudicaule*), wallflower (*Cheiranthus cheiri*), pansy (*Viola tricolor* L.), celery (*Apium graveolens*) var. Golden Self Blanching, common pimpernel or poor-man's-weatherglass (*Anagallis arvensis* L.), *Gilia tricolor*, tomato (*Lycopersicum esculentum* var. *vulgare*) var. Early Santa Clara Canner, green bell pepper (*Capsicum annuum* var. *grossum*), tobacco (*Nicotiana tabacum* L. var. Turkish), petunia (*Petunia hybrida*), snapdragon (*Antirrhinum majus*), nemesia (*Nemesia strumosa* Benth. var. *suttonii* Hort.), sunflower (*Helianthus annuus* L.), zinnia (*Zinnia elegans*) var. Double Lilliput Purple, English daisy (*Bellis perennis* L.), Michaelmas daisy (*Aster farreri* W. M. Sm., *A. horizontalis* hort. hybr. *grandiflorus*, *A. pyramidalis*, *A. subcoeruleus* S. Moore, and *A. yunnanensis* Franch.), French marigold (*Tagetes patula* L.), and African marigold (*T. erecta* L.).

Seeds of several species were planted in infested soil but no damping-off occurred on seedlings of cauliflower (*Brassica oleracea* var. *botrytis*) var. February, turnip (*B. rapa*) var. Purple Top White Globe, wallflower (*Cheiranthus cheiri*, celery (*Apium graveolens*), parsnip (*Pastinaca sativa*), *Gilia tricolor*, tomato (*Lycopersicum esculentum* var. *vulgare*), petunia (*Petunia hybrida*), snapdragon (*Antirrhinum majus*), zinnia (*Zinnia elegans*), dahlia (*Dahlia* sp.), and hybrid cineraria (*Senecio cruentus*).

Two varieties of China-aster (Giant Crego Azure Blue, wilt resistant, and Queen of the Market Lavender, wilt resistant) were readily infected by the isolate of *Phytophthora cryptogea* from annual stock after 17 days. However, the type culture from Pethybridge (host not specified), an isolate from African marigold (*18*), and the two isolates from Transvaal daisy did not cause infection after 41 days.

Plants of annual stock (variety Fiery Blood Red) were infected with the two isolates from Transvaal daisy after 23 days. The type culture yielded negative results.

Successful inoculations were made to Transvaal daisy plants with the isolate from annual stock, after 29 days, and with the isolate from marigold, after 22 days. The type culture did not cause infection.

SUMMARY

A foot rot of China-aster, annual stock, and Transvaal daisy, caused by *Phytophthora cryptogea* Pethybr. and Laff., is prevalent on the San Francisco peninsula and in other localities.

The principal environmental factors favoring the disease are excessive moisture, poor soil drainage, and cool weather.

Infected plants wilt very suddenly. The roots and the lower part of the stems of China-aster and annual stock and the roots and crowns of Transvaal daisy are involved in a soft, wet decay. Ultimately breakage of the stems or crowns at or near the soil level is followed by lodging and death of the plants.

The isolates of the fungus from China-aster and other hosts are described.

In the greenhouse, infection was obtained by adding the fungus to the wet, autoclaved soil of potted plants. The incubation period averaged 10 days for China-aster, 24 days for annual stock, and 45 days for Transvaal daisy.

The China-aster isolate was pathogenic to cucumber seedlings and young plants of wallflower, annual stock, Transvaal daisy, Michaelmas daisy, and hybrid cineraria, as well as to unwounded fruits of eggplant, ripe tomato, green bell pepper, pumpkin, watermelon, and cucumber.

The annual stock isolate caused infection of China-aster and Transvaal daisy.

When inoculated into annual stock, the Transvaal daisy isolates proved pathogenic, but no infection of China-aster was obtained.

An isolate of *Phytophthora drechsleri* from Transvaal daisy readily infected that host. Symptoms produced were indistinguishable from those induced by *P. cryptogea*.

No resistance to the disease was found in any of the commercial varieties of China-asters tested, including strains resistant to fusarium wilt.

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A FORMULA FOR REDUCING THE COMPUTATIONS NECESSARY TO FIND THE VARIANCE OF A SET OF AVERAGES [1]

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The yield of a three-row plot is often considered to be the average of the yields of the rows. The averages of these yields are used to obtain the mean and variance of the yields of the entire number of plots used in the experiment. Since the yield of each plot is an average of three-row yields, fractions are usually involved; also, deviations of the plot yields from the general mean of the group involve more fractions which increase the work of computation.

The weight of a steer is often considered to be the average of three measurements of his weight taken at different times during the day. Some research workers find the average of the three measurements, the deviations of these averages from the general average, and then the variance of the measurements from the sum of squares of these deviations. This method is used frequently in finding the variance of the weights of steers employed in a project. When this method of calculating the variance is employed, a great amount of computing is usually required in which decimal fractions arise.

The object of this paper is to show how to obtain the mean and variance of the measurements without the labor involved in finding the averages of the three measurements made at the outset and the deviations of these averages from the general mean.

Let x_1, y_1, z_1 represent, respectively, the yields of the three rows in the first plot; let x_2, y_2, z_2 represent the yields of the three rows in the second plot, etc. Yields of the plots are taken as the average of the three-row yields. They are, respectively,

$$W_1=\frac{x_1+y_1+z_1}{3},$$

$$W_2=\frac{x_2+y_2+z_2}{3},$$

$$W_3=\frac{x_3+y_3+z_3}{3},$$

$$\vdots \qquad \vdots$$

$$W_n=\frac{x_n+y_n+z_n}{3}.$$

The general mean, or the mean of all the plot yields, is

$$M=\frac{\Sigma W}{n}=\frac{\Sigma(x+y+z)}{3n}=\frac{\Sigma x+\Sigma y+\Sigma z}{3n},$$

which is the sum of all row yields divided by $3n$, the total number of rows in all plots.

[1] Received for publication Mar. 15, 1937; issued November, 1937. Journal Article no. 294 (n. s.) from the Michigan Agricultural Experiment Station.

It is not necessary to use the actual measurements, for by using an assumed or provisional mean the general mean and variance can be found. The use of a provisional mean enables one to reduce the size of the items and thus to lessen the chances of making errors. Let m be the provisional mean, which is usually some whole number near the center of the range of the measurements; let x_1' be the deviation of the yield of the first row in the first plot from the provisional mean, m; let y_1' be the deviation of the yield of the second row in the first plot from m, and z_1' the deviation of yield of the third row in the first plot from m; etc. The deviations of the original measurements from the provisional mean, m, are small numbers and hence are easier to use in computing.

The general mean, M, can be written in terms of the deviations from the provisional mean. By definition

$$\begin{aligned} x_1' &= x_1 - m,\ y_1' = y_1 - m,\ x_1' = z_1 - m \\ x_2' &= x_2 - m,\ y_2' = y_2 - m,\ z_2' = z_2 - m \\ &\vdots \quad\quad \vdots \quad\quad \vdots \quad\quad \vdots \\ x_n' &= x_n - m,\ y_n' = y_n - m,\ z_n' = z_n - m. \end{aligned}$$

Adding the above gives,

$$\Sigma(x_i' + y_i' + z_i') = \Sigma(x + y + z) - 3nm \tag{1}$$

or

$$\Sigma(x + y + z) = \Sigma(x_i' + y_i' + z_i') + 3nm$$

Hence

$$M = \frac{\Sigma(x+y+z)}{3n} = \frac{\Sigma(x'+y'+z')}{3n} + m \tag{2}$$

From the definition

$$x_i + y_i + z_i = x_i' + y_i' + z_i' + 3m \tag{3}$$

The variance [2] of the yields of the plots, obtained by taking the average of the three measurements, is by definition

$$\sigma^2 = \sum_{i=1}^{n}\left(\frac{x_i+y_i+z_i}{3} - \sum_{i=1}^{3n}\left(\frac{x_i+y_i+z_i}{3n}\right)\right)^2 \Big/ (n-1)$$

or

$$\sigma^2 = \sum_{i=1}^{n}\left(\frac{n(x_i+y_i+z_i) - \sum_{i=1}^{3n}(x_i+y_i+z_i)}{3n}\right)^2 \Big/ (n-1) \tag{4}$$

Substituting the values of $\Sigma(x_i+y_i+z_i)$ and $x_i+y_i+z_i$ from (2) and (3) respectively into (4), gives

$$\sigma^2 = \sum_{i=1}^{n}\left(\frac{n(x_i'+y_i'+z_i') + 3nm - \sum_{i=1}^{3n}(x_i'+y_i'+z_i') - 3nm}{3n}\right)^2 \Big/ (n-1) \tag{5}$$

$$\therefore\ \sigma^2 = \frac{1}{(n-1)}\sum_{i=1}^{n}\left(\frac{n(x_i'+y_i'+z_i') - \sum_{i=1}^{3n}(x_i'+y_i'+z_i')}{3n}\right)^2.$$

[2] This is considered to be the best estimate of the variance of the population from which the sample is taken.

Now, carrying out the summation in the above line, we have the following without the denominators

$$n^2(x_1'+y_1'+z_1')^2-2n\Sigma(x_j'+y_j'+z_j')(x_1'+y_1'+z_1')+(\Sigma(x_j'+y_j'+z_j'))^2$$
$$n^2(x_2'+y_2'+z_2')^2-2n\Sigma(x_j'+y_j'+z_j')(x_2'+y_2'+z_2')+(\Sigma(x_j'+y_j'+z_j'))^2$$
$$\vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots$$
$$n^2(x_n'+y_n'+z_n')^2-2n\Sigma(x_j'+y_j'+z_j')(x_n'+y_n'+z_n')+(\Sigma(x_j'+y_j'+z_j'))$$

Adding, we get

$$n^2\sum_{i=1}^{n}(x_i'+y_i'+z_i')^2-2n\sum_{j=1}^{n}(x_j'+y_j'+z_j')\sum_{j=1}^{n}(x_j'+y_j'+z_j')+n\left(\sum_{j=1}^{n}(x_j'+y_j'+z_j')\right)^2$$

$$\therefore\ \sigma^2=\left\{n^2\sum_{i=1}^{n}(x_i'+y_i'+z_i')^2-n\left(\sum_{i=1}^{n}(x_j'+y_j'+z_j')\right)^2\right\}\Big/9n^2(n-1)$$

or

$$(6)\qquad \sigma^2=\left\{n\sum_{i=1}^{n}(x_i'+y_i'+z_i')^2-\left(\sum_{j=1}^{n}(x_j'+y_j'+z_j')\right)^2\right\}\Big/9n(n-1)$$

Formula (6) looks complicated; actually it is very easy to use, as table 1 concerning yields of three-row plots of Minhardi wheat will show. The first three columns in table 1 contain wheat yields for the three rows of 10 plots. The next three columns contain the deviations of the yields from the provisional mean, 250 g. In column 7 are recorded the sum of the items in columns 4, 5, and 6; the last column contains the squares of the numbers in column 7.

TABLE 1.—*Data to illustrate the use of formula (6)*

Yield of Minhardi wheat			$x-250$	$y-250$	$z-250$	$x'+y'+z'$	$(x'+y'+z')^2$
Row 1 x	Row 2 y	Row 3 z	x'	y'	z'		
Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
247	251	224	−3	1	−26	−28	784
249	223	233	−1	−27	−17	−45	2,025
271	252	221	21	2	−29	−6	36
263	233	255	13	−17	5	1	1
280	215	260	30	−35	10	5	25
255	234	258	5	−16	8	−3	9
256	217	241	6	−33	−9	−36	1,296
262	226	268	12	−24	18	6	36
297	267	284	47	17	34	98	9,604
288	252	269	38	2	19	59	3,481
Total						+51	17,297

From (2) the value of the general mean is $M=250+\frac{51}{30}=251.7$ g.

From formula (6) the variance of this set of data, where the plot yield is considered to be the average of the yields of the three rows in the plot, is

$$\sigma^2=\frac{10(17,297)-(51)^2}{90\ (9)}=\frac{170,369}{90(9)}=210.3$$

$$\therefore \sigma=\sqrt{210.3}=14.5\text{g}$$

In the foregoing illustration there were no fractions in the calculations until the last step in finding M and the last division in finding σ^2.

A similar formula can be derived from n plots, each with k rows, where the yield of a plot is considered to be the average of the yields of the k rows. The formulas for mean and variance are

$$M=\frac{\sum_{i=1}^{n}(a_i'+b_i'+c_i'+\cdots+k_i')}{k\cdot n}+m \tag{7}$$

and

$$\sigma^2=\frac{n\sum_{i=1}^{n}(a_i'+b_i'+c_i'+\cdots+k_i')^2-\left(\sum_{i=1}^{n}(a_i'+b_i'+c_i'+\cdots+k_i')\right)^2}{k^2\cdot n(n-1)} \tag{8}$$

where a_i is the yield of the first row of the ith plot, and a_i' is the deviation of the yield of the first row in the ith plot from the provisional mean m.

Let us now consider a set of weight measurements made on 13 steers, where the weight of a steer is considered to be the average of five measurements made during the first day of the feeding experiment, as shown in table 2.

TABLE 2.—*Data to illustrate the use of formulas (7) and (8)*

Steer no.	Weights of steers taken at 5 times during first day					$m=310$					(h) $a'+b'+c'+d'+e'$	$(h)^2$
	First a	Second b	Third c	Fourth d	Fifth e	$a-310$ a'	$b-310$ b'	$c-310$ c'	$d-310$ d'	$e-310$ e'		
	Lb.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1	300	315	304	308	310	−10	5	−6	−2	0	−13	169
2	285	280	293	297	288	−25	−30	−17	−13	−22	−107	11,449
3	325	322	317	329	320	15	12	7	19	10	63	3,969
4	337	324	330	334	328	27	14	20	+24	18	103	10,609
5	295	307	301	299	306	−15	−3	−9	−11	−4	−42	1,764
6	271	283	280	278	285	−39	−27	−30	−32	−25	−153	23,409
7	305	319	312	318	306	−5	9	2	8	−4	10	100
8	329	322	314	319	324	19	12	4	9	14	58	3,364
9	304	309	317	321	314	−6	−1	7	11	4	15	225
10	325	333	335	340	330	+15	23	25	30	20	113	12,769
11	301	313	297	303	307	−9	3	−13	−7	−3	−29	841
12	279	288	291	284	275	−31	−22	−19	−26	−35	−133	17,689
13	298	294	306	301	290	−12	−16	−4	−9	−20	−61	3,721
Total											−176	90,078

From (7) the mean of the weights, where weight is considered to be the average of the five weights made during the first day, is

$$M=\frac{(-176)}{5\times 13}+310=307.29 \text{ pounds.}$$

From (8) the variance is

$$\sigma^2=\frac{13(90{,}078)-(-176)^2}{25\times13\times12}=292.32$$

and

$$\sigma=17.1 \text{ pounds.}$$

This formula may be employed to shorten computation work when Harris' formula is used for finding the intraclass correlation coefficient. Harris' [3] formula is

$$k\sum_{p=1}^{n'}(\bar{x}_p-\bar{x})^2=ns^2.[1+(k-1)n], \tag{9}$$

where k=the number of items in the same family; n'=the number of families;
$\bar{x}$=the mean of all items; n=the number of degrees of freedom;
s^2=the variance of an entire set of items; $\bar{x}_p$=the mean of the items in the p th family: r=the intraclass correlation.

The summation, $\sum_{p=1}^{n'}(\bar{x}_p-\bar{x})^2$, in formula (9) can be found by formula (6), as the following example shows.

The number of pedicels per inflorescence of nonterminal inflorescences on the second branch of a certain plant were counted for obtaining the correlation between number of pedicels for sister clusters. There were three nonterminal inflorescences on the second branch of these plants. Table 3 gives the data.

TABLE 3.—*Data to illustrate the use of formulas (6) and (9)*

Pedicels per cluster			$m=8$				
First cluster x	Second cluster y	Third cluster z	x'	y'	z'	$x'+y'+z'$	$(x'+y'+z')^2$
Number	*Number*	*Number*					
8	9	8	0	1	0	1	1
10	10	9	2	2	1	5	25
7	8	7	−1	0	−1	−2	4
9	7	8	1	−1	0	0	0
6	7	6	−2	−1	−2	−5	25
8	9	9	0	1	1	2	4
8	8	8	0	0	0	0	0
7	7	6	−1	−1	−2	−4	16
10	9	8	2	1	0	3	9
8	9	9	0	1	1	2	4
7	6	8	−1	−2	0	−3	9
11	8	9	3	0	1	4	16
9	9	9	1	1	1	3	9
8	8	8	0	0	0	0	0
7	6	6	−1	−2	−2	−5	25
9	8	8	1	0	0	1	1
7	7	7	−1	−1	−1	−3	9
11	11	10	3	3	2	8	64
8	8	7	0	0	−1	−1	1
10	8	10	2	0	2	4	16
Total						−6	238

[3] Harris, J. A. A CONTRIBUTION TO THE PROBLEM OF HOMOTYPOSIS. Biometrika 11: 201–214, illus. 1916.

By using the quantity in brackets in formula (6) the sum, $\Sigma(\bar{x}_p - \bar{x})^2$, is equal to

$$\Sigma(\bar{x}_p - \bar{x})^2 = \frac{20(238) - (6)^2}{9 \cdot 20} = \frac{4724}{180}.$$

This value used in formula (9) leads to an intraclass correlation coefficient of 0.925.

Formulas (7) and (8) enable one to find the mean and the standard deviation of a set of averages without actually finding the averages or the deviations from the general mean and by employing smaller numbers than the original measurements. These formulas reduce the work of computation.

THE URONIC ACID CONTENT OF THE NITROGEN-FREE EXTRACT OF FEEDING STUFFS[1]

By G. A. GUANZON, *instructor in sugar technology, college of agriculture, Laguna, P. I.*, and W. M. SANDSTROM, *assistant chemist, Minnesota Agricultural Experiment Station*

INTRODUCTION

Abundant data exist on the percentage of nitrogen-free extract in feeds, but in only a few instances is there information concerning the kind and quantity of carbohydrates comprising it. According to their digestibility, Fraps (*6*)[2] arranged the known components of the nitrogen-free extract in the following order: (1) Sugars, (2) starch, (3) pentosans, (4) residue. It is also being recognized that the effects of association of the different components may have a great deal to do with digestibility. Thus, results (*5*, *6*, *7*, *8*, *9*, *13*, *14*) show that the sugars and starches and the pentosans of hays and forages are generally less digestible than the same constituents in starchy concentrates and meal feeds.

The term "residual nitrogen-free extract" is given by Fraps (*10*) to that part of the nitrogen-free extract remaining after the values for sugars, starch, and pentosans have been subtracted. This fraction includes the uronic acids, with the determination of which this paper deals.

Polymers of uronic acids are widespread in the plant world as incrusting materials and as constituents of cell walls, and in the hemicellulose and pectic materials. Link (*15*, *16*) has demonstrated the presence of free glycuronic acid in corn seedlings. It is well known that compounds like benzoic acid and certain terpene derivatives are eliminated from the higher animals as "paired glycuronic acids." Mathews (*17*) suggests that the uronic acid is a transitory stage in the oxidation of glucose in the body; however, it has never been shown that the higher animals are able to utilize the uronic complexes.

METHODS OF EXPERIMENTATION

MATERIALS

Nine feeds representing concentrates, grain byproducts, and roughage were selected to give a variety with respect to physical and chemical characteristics, as is shown in table 1. The peanuts were purchased in the open market; the other materials were available at the Minnesota Agricultural Experiment Station. All were obtained in an air-dried condition. In each case a 300-g sample was coarsely ground and then placed in a ball mill until the particles were reduced to a size passing through a sieve having circular openings of 1 mm. diameter.

[1] Received for publication June 11, 1936; issued November 1937. Contribution no. 1428 of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 585.

TABLE 1.—*Composition of feeding stuffs (moisture free)*

Feed	Crude fiber	Pentosans in crude fiber	Nitrogen-free extract	Uronic acid anhydride	Sugars and starch			
					Reducing sugars	Sucrose	Starch	Total
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Alfalfa hay	30.31	5.03	40.37	10.40	1.76	1.65	1.44	4.85
Corncobs	36.53	4.48	58.87	5.88	1.44	.60	2.03	4.07
Corn bran	16.96	1.16	62.46	6.64	.30	nil	7.34	7.64
Peanut hulls	76.40	14.47	14.70	6.00	.83	.48	.88	2.11
Peanut kernels	2.43	.20	14.95	1.92	(1)	5.39	3.27	8.66
Rye	2.21	.15	81.36	.72	.17	5.43	53.68	59.28
Timothy hay	34.55	5.98	47.19	5.00	3.57	1.39	.74	5.70
Wheat bran	12.11	.99	53.23	3.88	.70	3.91	7.83	12.44
Wheat shorts	6.74	.49	68.96	1.68	1.16	4.34	20.73	26.23

[1] Trace.

ANALYTICAL METHODS

The uronic acid content was determined by the method of Dickson, Otterson, and Link (*4*) except that the liberated carbon dioxide was received in two spiral tubes (Bailey (*3*)) in series, and the excess of barium hydroxide titrated in these containers. Approximately 20 minutes of heating is required to bring the temperature of the reaction mixture to 100° C., during which time any carbon dioxide evolved from carbonates present is removed. At this stage the standardized solution of barium hydroxide is introduced into the receiving train. This is a slight modification of Anderson's method (*1*). Norris and Resch (*18*) have recently verified the statement that the determination of uronic acids by decarboxylation is quantitative. In the case of the rye, wheat shorts, and wheat bran samples, which contain large quantities of starch and sugars, correction was made for the carbon dioxide evolved under the conditions of the determination (0.45 percent of the weight of the carbohydrate). The quantities of protein present do not yield a measurable amount of carbon dioxide under the above conditions.

The determinations of moisture, ash, crude protein, pentosans, sucrose, reducing sugars, starch, and ether extract were made by the official methods of the Association of Official Agricultural Chemists (*2, pp. 335, 336, 25, 344, 341, 480, 342, and 339*, respectively). Crude fiber was determined by the Kennedy modification (*11*). Pentosans were also determined on a sample treated for weighing as crude fiber and both pentosans and crude protein determined on a fat-free sample which had been extracted for 30 minutes with boiling N/50 sulphuric acid, followed by 15 minutes of boiling after the addition of enough sodium hydroxide to neutralize the acid and give a solution N/50 with regard to the alkali (*7*). Only part of these values are reported in this paper, however their determinations were necessary in order to calculate the values for the nitrogen-free extract.

CALCULATIONS

Pentosans in nitrogen-free extract.—The differences between the total pentosans and the pentosans in crude fiber are taken to be the pentosans in the nitrogen-free extract.

Residual nitrogen-free extract.—The percentages of sugars, starch, and pentosans in the nitrogen-free extract were added together and the sum subtracted from the total nitrogen-free extract. The remainder is termed the residual nitrogen-free extract.

Insoluble pentosans in nitrogen-free extract.—The pentosans were determined on the residue insoluble in N/50 acid and alkali. The pentosans in the crude fiber as determined previously were subtracted, and the remainder is termed the insoluble pentosans in nitrogen-free extract. The soluble pentosans in the nitrogen-free extract were calculated by differences.

Insoluble residual nitrogen-free extract.—From the value for the residue insoluble in N/50 acid and alkali was subtracted the sum of the value for crude fiber plus the values for the crude protein and the pentosans in that insoluble residue. The difference is termed the insoluble residual nitrogen-free extract. The value for the soluble residual nitrogen-free extract was calculated by differences.

DATA

Table 1 gives the composition of the feeds on the moisture-free basis; all values are the average of three determinations. Table 2 records the composition of certain constituents of the nitrogen-free extract calculated as percentages of that fraction.

TABLE 2.—*Composition of the nitrogen-free extract of feeding stuffs*

Feed	Uronic acid anhydrides	Sugars and starch	Pentosans			Residual nitrogen—free extract		
			Soluble	Insoluble	Total	Soluble	Insoluble	Total
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Alfalfa hay	25.76	12.01	17.24	8.27	25.51	29.67	32.83	62.50
Corncobs	9.99	6.91	19.20	33.55	52.74	6.13	34.20	40.33
Corn bran	10.63	12.23	41.10	8.92	56.45	18.24	13.08	31.32
Peanut hulls	41.50	14.35	6.87	34.15	40.68	9.59	34.90	44.49
Peanut kernels	12.84	57.93	15.25	6.29	21.54	14.65	5.81	20.46
Rye	.88	72.86	9.77	2.14	11.91	11.37	3.86	15.23
Timothy hay	10.60	12.08	2.61	26.30	28.90	29.75	29.24	58.99
Wheat bran	7.29	23.37	23.61	20.72	44.34	16.23	16.06	32.29
Wheat shorts	2.44	38.04	14.16	7.57	21.74	31.95	8.27	40.22

DISCUSSION

In general the uronic acid content only partially tends to parallel the crude fiber content; in the case of the two hays and the corncobs the values are out of line. The pentosan content of the crude fiber shows a better agreement with the total crude fiber, thus suggesting that the variations in the uronic acid content are to be ascribed to the portion found in the nitrogen-free extract.

In comparing the values obtained for the various constituents in the nitrogen-free extract it is to be recognized that the "pentosan" values will include the pentose moiety of the uronic acid anhydrides. On the basis of equivalent weights 75 percent of the uronic acid anhydride is pentose, but Norris and Resch (*18*) have shown that the uronic acids yield only approximately 42 percent of the theoretical amount of furfural. On this basis 31.5 percent of the weight of the uronic acid anhydrides is included in the "pentosan" fraction as determined. The appropriate deductions from the total pentosan in the nitrogen-free extract have been made to give the corrected pentosan values. Only in the case of the alfalfa hay and the peanut hulls

is the change very great. The corrected pentosan value of the former is 17.41 percent as contrasted with 25.51 percent of the nitrogen-free extract, while in the case of the peanut hulls the value becomes 27.62 percent instead of 40.68 percent. Since it is not known how the uronic acids are distributed as between soluble and insoluble "pentosans", and since the corrections are slight (ranging in difference from 0.3 to 3.3 percent), the corrected values for the other feeds are not shown.

An examination of table 1 fails to reveal any very great regularity between the uronic acid content and the total nitrogen-free extract of the feeds examined, although there is a tendency for the two to vary inversely. As is to be expected, the concentrates contain higher percentages of starch and the soluble sugars, and with the exception of the peanut kernels, these latter vary inversely with the uronic acid content. Among the roughages the starches and sugars occur at about the same lower level but associated with higher concentrations of uronic acids. The proportionalities between the various known constituents of the nitrogen-free extracts are shown more clearly by the method of calculation employed in table 2.

The residual nitrogen-free extract contains the miscellaneous products; of these the uronic acid anhydrides in the feeds are here determined for the first time. There is no marked tendency to regularity between the content of uronic acids and the total residual extract. Except in the case of corn bran and peanut kernels, the uronic acid content of these feeds increases with the insoluble residual nitrogen-free extract, but no regularity appears when the soluble portion is considered.

Since the pentosan fractions as determined should include that arising from the uronic acids present, as pointed out earlier; and since pectins, hemicelluloses, and gums contain uronic acids (*4*, *5*, *7*); and since the pectins and gums may be sufficiently soluble to escape the crude fiber determination (*5*), we might expect to find some relationship between the uronic acids and pentosans. There is, again, no relationship to be observed when the soluble and the insoluble pentosans are separately compared with the uronic acid content of each feed.

The digestibility of the total nitrogen-free extract is no doubt the resultant of the digestibility of the individual components of this fraction. Investigators, particularly Fraps (*10*), have referred the lower values of the digested matter of roughages as compared with those of concentrates to the presence of unknown and undetermined constituents. Fraps points out that the "pentosans soluble in N/50 acid and alkali are digested to a greater extent than the remaining pentosans" (*7*) and that "the nitrogen-free extract soluble in N/50 acid and alkali is, as a rule, digested to a greater extent than that not soluble" (*8*). With this in mind the calculated percentages of digestibility of the feeds as shown in table 3 were made by assuming that the starches and sugars were 100 percent utilized, and that the pentosans and the residual nitrogen-free extract were utilized to the extent that they were soluble in N/50 acid and alkali. The calculated digestibility was arrived at by giving effect to the proportions of the various constituents. This calculation was made in order to compare the results with those obtained for the nitrogen-free extract from feeding trials as summarized by Henry and Morrison (*12, appendix table II*).

TABLE 3.—*Digestibility of feeds and of their nitrogen-free extract as related to uronic acid content of each*

Feed	Uronic acid content of—		Calculated digestibility of total nitrogen-free extract	Coefficients of digestibility from feeding trials (Henry and Morrison (12))
	Feeds	Nitrogen-free extract		
	Percent	*Percent*	*Percent*	
Alfalfa hay	10.40	25.76	58.92	70–72
Corncobs	5.88	9.99	32.24	52
Corn bran	6.64	10.63	71.57	80
Peanut hulls	6.00	41.50	30.81	----
Peanut kernels	1.92	12.84	87.83	84
Rye	.72	.88	94.00	88–94
Timothy hay	5.00	10.60	44.44	47–62
Wheat bran	3.88	7.29	63.21	66–72
Wheat shorts	1.68	2.44	84.15	78–88

The calculated digestibilities agree surprisingly well with results obtained by actual feeding trials. There is a closer agreement in the case of the concentrates with their high content of starches and sugars and the lower content of uronic acid and residual nitrogen-free extract. On the other hand, the calculated digestibilities are somewhat lower than those obtained by feeding trials in the case of the roughages which contain a greater amount of uronic acid anhydrides. These latter are only part of the residual nitrogen-free extract, but the results suggest that the lowered digestibility may in part be accounted for by the uronic acids.

SUMMARY

Analyses on nine feeds classified as concentrates, grain byproducts, and roughages have been made in the usual way but to include uronic anhydrides in the residual nitrogen-free extract. Roughages in general contain a higher percentage of the uronic acid anhydrides than do the concentrates. There does not appear to be any regularity between the uronic anhydride content and the other constituents of the nitrogen-free extract. A calculated artificial coefficient of digestibility of these feeds compares fairly well with the results of feeding trials, but the parallelism is less close in the feeds with a high nitrogen-free extract, part of which is of a uronic acid nature.

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THE EFFECT OF MECHANICAL PROCESSING OF FEEDS ON THE MASTICATION AND RUMINATION OF STEERS [1]

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INTRODUCTION

Numerous data are available in the literature regarding the effects which various methods of preparation of concentrates and roughages have on the utilization of these feeds by cattle. The conditions under which the different trials have been conducted are so variable from the standpoint of the type of animal, indices used, and the degree of control of the many factors involved that it is difficult to draw conclusions from the results reported.

If mechanical processing of feeds is of any value other than increasing palatability or decreasing waste, it would appear that it must certainly save the animal some energy in the comminution of the feed by reducing the amount of work required in the processes of mastication and rumination. If this be true, the energy required for the so-called work of digestion would be reduced and the metabolizable energy so conserved should be available for production purposes.

REVIEW OF THE LITERATURE

Bergman and Dukes [2] reported a critical study on two cows fed a normal dairy ration of grain, silage, and hay. They found that one animal spent 6 hours and 42 minutes and the other spent 8 hours and 22 minutes daily in rumination. In the case of each animal this process occurred in 15 to 20 periods which were evenly distributed over the entire day. The length of these periods varied from 2 to 49 minutes with an average of 26 minutes. The total number of boluses regurgitated paralleled the total number of minutes of rumination. Approximately 59 seconds was devoted to each bolus and about 5 seconds of this time was occupied by the processes of rumination other than remastication and reinsalivation. No correlation between the amounts of feed consumed and the time spent in rumination was reported.

Fuller [3] obtained detailed information on three dairy cows fed a normal ration. He reported averages of 5 hours 57 minutes eating, 8 hours 5 minutes ruminating, 53.9 seconds remasticating each bolus, and 3.73 seconds spent in deglutition and regurgitation. Twenty-two cows in stanchions daily spent approximately 3 hours eating, 8 hours ruminating, and made approximately 41,000 jaw movements daily. The rate of the jaw movements depended on the kind of material being chewed—94 per minute for the mastication of grain and silage, 78 per minute for the mastication of hay, and 55 per minute during rumination.

[1] Received for publication Mar. 29, 1937; issued November 1937.

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Schalk and Amadon [4] reported data on the time required for the mastication of various feeds by dairy cows. They found that the following lengths of time were required for 5-pound allowances of the various feeds: Whole oats 10 minutes, corn on the cob 18 minutes, ground feed 14 minutes, hay 15 minutes.

The same authors [4] obtained results of a study of rumination with nine dairy cows on rations of ground grain, alfalfa hay, and corn silage. The animals were followed continuously for 60 hours. The observations obtained, based on a 24-hour period, were 6.14 hours occupied in eating, 6.89 hours ruminating, and 10.90 hours resting.

Four animals were observed continuously for 60 hours for more detailed information,[4] and it was found that the total time spent in rumination was divided into 162 periods including 66 with the animal in the standing position, 75 in the lying position, and 21 in both positions for part of the period. The average number of boluses per period was 25.37, with 50.32 chews being made on each bolus. During the entire period of 60 hours the four animals regurgitated 4,110 boluses and made 206,663 chews.

Two animals were followed in detail for 24 hours while on pasture during the summer.[4] Thirty rumination periods were observed to average 26.1 boluses per period and the average number of chews per bolus was 39.11. Sixteen of these periods occurred while the animals were standing and 14 while lying. They regurgitated 793 boluses and made 31,013 jaw movements during the 24 hours.

The authors concluded that such factors as age, condition of the teeth, and the nature and kind of feed all affected rumination. Flies, mosquitoes, dogs, interference by herd mates, or any condition which would worry, frighten, or disturb the animal may lead to a cessation of rumination. If the stoppage was momentary, the animal would hold the bolus in the oral cavity and presently would resume chewing. However, the number of chews was always lessened and the total time of rumination decreased.

The present investigations were undertaken as part of a larger program on the study of digestion in cattle. They were planned to determine the effects of the processing of both concentrates and roughages on mastication and rumination in steers of various ages.

METHOD OF PROCEDURE

The animals used were ordinary feeder steers which had been purchased on the open market. They had all been operated on to produce rumen fistulas for purposes other than the experiments to be reported. All the steers were in good physical condition but in rather thin flesh during these trials. Their ages at the beginning of the tests varied from 17 to 31 months.

The following rations were fed to each steer:

Ration 1, whole alfalfa hay.

Ration 2, coarsely cut alfalfa hay—modulus 4.55—uniformity 5:5:0.[5]

[4] SCHALK, A. F., and AMADON, R. S. PHYSIOLOGY OF THE RUMINANT STOMACH (BOVINE); STUDY OF THE DYNAMIC FACTORS. N. Dak. Agr. Expt. Sta. Bull. 216, 64 pp., illus. 1928.

[5] The first expression of figures is the modulus of fineness; the second expression is the uniformity of the material in regard to size of particles. The fineness of the material was made by the modulus system. Seven screens are used which range from a ⅜-inch mesh to 100 meshes per inch. This series of screens separates the material into various particle sizes. By weighing what remains on each screen a figure is computed which gives the fineness of the material. The larger number denotes coarse material and the smaller number denotes fine material. The consistency of uniformity of the material was arrived at by adding the percentage of material remaining on the coarse screens, the medium screens, and the fine screens. This gives a ratio of three figures which denotes the relationship in the amounts of coarse, medium, and fine material in any given sample.

Ration 3, finely cut alfalfa hay—modulus 3.55—uniformity 1:7:2.
Ration 4, ground alfalfa hay—modulus 3.25—uniformity 1:6:3.
Ration 5, whole alfalfa hay, protein supplement—modulus 2.01—uniformity 0:3:7, shelled yellow corn—modulus 6.01—uniformity 10:0:0.
Ration 6, whole alfalfa hay, protein supplement—modulus 2.01—uniformity 0:3:7, ground shelled yellow corn—modulus 3.56—uniformity 2:6:2.
Ration 7, protein supplement—modulus 2.01—uniformity 0:3:7; shelled yellow corn—modulus 6.01—uniformity 10:0:0.

A good-quality alfalfa hay was used throughout the trials. The coarsely cut and finely cut hays were approximately 2-inch and ¼-inch pieces which had been cut in an ordinary hay cutter. Figure 1 shows samples of the alfalfa hay as fed. The hay and corn were ground by a hammer-type mill. The protein supplement consisted of a mixture

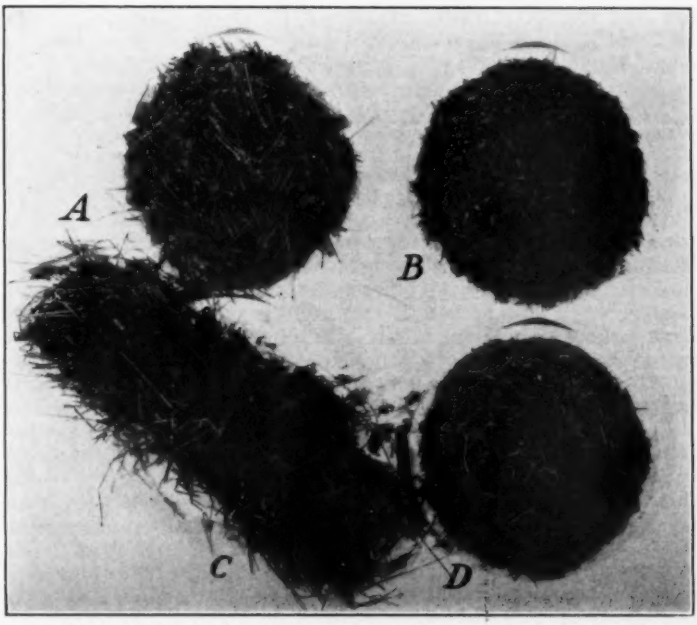

FIGURE 1.—Samples of the various hays fed: *A*, coarsely cut alfalfa, approximately 2-inch lengths; *B*, finely cut alfalfa, approximately ¼-inch lengths, *C*, whole alfalfa; *D*, ground alfalfa.

of dry-rendered tankage, cottonseed, linseed, and soybean-oil meals, steamed bonemeal, limestone, and salt. Block salt was before the animals at all times.

The rations were fed at approximately 8 a. m. and 4 p. m. in such amounts as the animals would consume readily. Each steer was given exactly the same amount of the various kinds of hay in order to have a common basis on which to compare the amounts of mastication and rumination. The corn and hay of the mixed rations were always fed in the ratio of 3 parts of corn to 1 part of hay, the amount of corn being regulated by the appetite of the animal. The shelled corn in ration 5 was replaced by an equivalent weight of ground corn in ration 6. In the last trial (ration 7), the roughage was omitted from the feed, the

amount of protein supplement was maintained as in the two previous trials, and the animals were allowed all the shelled corn they would consume.

A preliminary period of a week, in some cases more, was introduced between trials, during which time the animals were fed the rations to be tested during the following period. This preliminary period served to accustom the animals to the new feed and also to remove any residue of the preceding ration which might remain in the rumen or reticulum.

Each trial was begun at approximately 8 a. m. when the animals were given their morning feed. The number of jaw movements was recorded by means of mechanical counters and the time taken was noted. This process was repeated about 4 p. m., when the evening feed was offered. During the remainder of the 24-hour period the steers were under constant surveillance, and the time, number of chews, number of boluses regurgitated, and the number of chews per bolus during the entire daily rumination were recorded. Each trial was continued for 48 consecutive hours, and the data presented represent, in each case, an average of two 24-hour periods.

OBSERVATIONS

In general, as the finer hay was fed the number of chews taken for mastication was reduced in all the animals regardless of age (table 1). There was no apparent difference in the amount of mastication required for the long and the coarsely cut hay in the case of the youngest steer (A). Steer D did not relish the ground hay and minced about while eating, which would account for the increased number of jaw movements taken for the mastication of this material. All of the animals took more chews in the mastication of ground corn than shelled corn. The number of chews per unit weight of feed decreased as the age of the animal and the amount of feed consumed increased.

The total time spent by each steer in the mastication of the different rations (fig. 2) varied directly with the total number of chews made. When the time required per unit of feed was calculated it was apparent that the younger animals (A and E) spent more time in masticating their feed than the older animals (B and D). The percent of the daily time spent in mastication varied from 4.3 to 11.0 depending on the fineness of the material eaten. In the case of the various roughages, the amount of time decreased as the size of the particles decreased. The reverse was true in the case of the corn, shelled corn requiring less time for mastication than did ground corn. The only obvious reason for the discrepancy in the time required for the mastication of whole alfalfa when fed alone and in combination with the concentrates is the fact that on the higher plane of nutrition the appetites of the animals were keener. The rate of chewing was variable but could not be correlated with the physical condition of the feed.

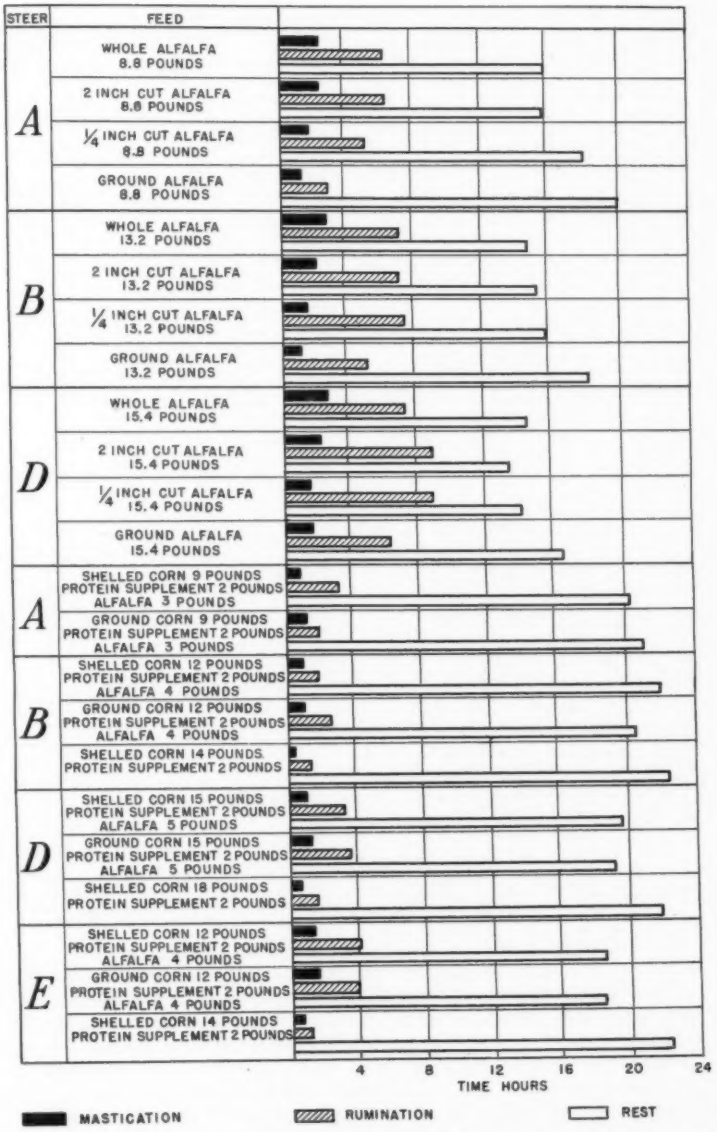
STEER
FEED
A
WHOLE ALFALFA 8.8 POUNDS
2 INCH CUT ALFALFA 8.8 POUNDS
¼ INCH CUT ALFALFA 8.8 POUNDS
GROUND ALFALFA 8.8 POUNDS
B
WHOLE ALFALFA 13.2 POUNDS
2 INCH CUT ALFALFA 13.2 POUNDS
¼ INCH CUT ALFALFA 13.2 POUNDS
GROUND ALFALFA 13.2 POUNDS
D
WHOLE ALFALFA 15.4 POUNDS
2 INCH CUT ALFALFA 15.4 POUNDS
¼ INCH CUT ALFALFA 15.4 POUNDS
GROUND ALFALFA 15.4 POUNDS
A
SHELLED CORN 9 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 3 POUNDS
GROUND CORN 9 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 3 POUNDS
B
SHELLED CORN 12 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 4 POUNDS
GROUND CORN 12 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 4 POUNDS
SHELLED CORN 14 POUNDS PROTEIN SUPPLEMENT 2 POUNDS
D
SHELLED CORN 15 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 5 POUNDS
GROUND CORN 15 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 5 POUNDS
SHELLED CORN 18 POUNDS PROTEIN SUPPLEMENT 2 POUNDS
E
SHELLED CORN 12 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 4 POUNDS
GROUND CORN 12 POUNDS PROTEIN SUPPLEMENT 2 POUNDS ALFALFA 4 POUNDS
SHELLED CORN 14 POUNDS PROTEIN SUPPLEMENT 2 POUNDS
4
8
12
16
20
24
TIME HOURS
MASTICATION
RUMINATION
REST

FIGURE 2.—Total time spent by steers in masticating, ruminating, and resting during a 24-hour period. Age of animals: A, 17 to 28 months; B, 22 to 33; D, 31 to 42; and E, 28 months

TABLE 1.—*Effect of various methods of preparation of alfalfa hay and of grinding corn on mastication by steers*

[Data are for two 24-hour periods]

ALFALFA HAY

Steer	Age	Quantity and preparation of ration	Mastication					
			Chews	Chews per pound	Total time	Time per pound	Portion of day spent in mastication	Chews per minute
	Months		*Number*	*Number*	*Minutes*	*Minutes*	*Percent*	*Number*
A	17	8.8 pounds whole alfalfa	10,298	1,170	141	16.0	9.8	73
		8.8 pounds 2-inch cut alfalfa	10,686	1,214	143	16.3	9.9	75
	19	8.8 pounds ¼-inch cut alfalfa	6,737	766	99	11.3	6.9	68
		8.8 pounds ground alfalfa	5,609	637	76	8.6	5.3	74
B	22	13.2 pounds whole alfalfa	13,085	991	159	12.0	11.0	82
		13.2 pounds 2-inch cut alfalfa	10,280	779	121	9.2	8.4	85
	24	13.2 pounds ¼-inch cut alfalfa	6,182	468	84	6.4	5.8	74
		13.2 pounds ground alfalfa	4,556	345	62	4.7	4.3	73
D	31	15.4 pounds whole alfalfa	13,935	905	158	10.3	11.0	88
		15.4 pounds 2-inch cut alfalfa	9,824	638	126	8.2	8.8	78
	33	15.4 pounds ¼-inch cut alfalfa	7,060	458	90	5.8	6.3	78
		15.4 pounds ground alfalfa	8,330	541	96	6.2	6.7	87

GROUND CORN

A	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds shelled corn	1,723	574	26	8.7	1.8	66
			2,031	185	26	2.4	1.8	78
	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds ground corn	2,054	685	28	9.3	1.9	73
			2,667	242	36	3.3	2.5	74
B	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	2,643	661	35	8.8	2.4	76
			1,721	123	20	1.4	1.4	86
	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	2,423	606	32	8.0	2.2	76
			2,232	159	25	1.8	1.7	89
	36	2 pounds protein supplement, 14 pounds shelled corn	1,872	117	22	1.4	1.5	85
D	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds shelled corn	2,719	544	38	7.6	2.6	72
			2,429	143	30	1.8	2.1	81
	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds ground corn	2,850	570	39	7.8	2.7	73
			3,159	186	36	2.1	2.5	88
	42	2 pounds protein supplement, 18 pounds shelled corn	3,012	151	37	1.9	2.6	81
E	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	3,653	913	53	13.2	3.7	69
			2,202	157	33	2.4	2.3	67
	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	3,987	997	51	12.7	3.5	78
			3,400	243	42	3.0	2.9	81
	28	2 pounds protein supplement, 14 pounds shelled corn	2,699	169	36	2.3	2.5	75

The effects of processing feed on rumination are presented in table 2. Cutting the alfalfa into lengths as short as one-fourth inch had no marked effect on the number of chews, the time taken for rumination (fig. 2), the number of periods this time was divided into (fig. 3), the number of boluses regurgitated, or the number of chews made and the time spent on each bolus. However, grinding the hay reduced the number of chews and the time required for rumination and this

reduced the number of chews made per pound of feed. The daily time spent in rumination of the whole and cut hays averaged approximately 29 percent, while in the case of the ground hay this was reduced to 19 percent. The youngest animal (A) evidenced a slight reduction in the number of chews and the time required for rumination on the

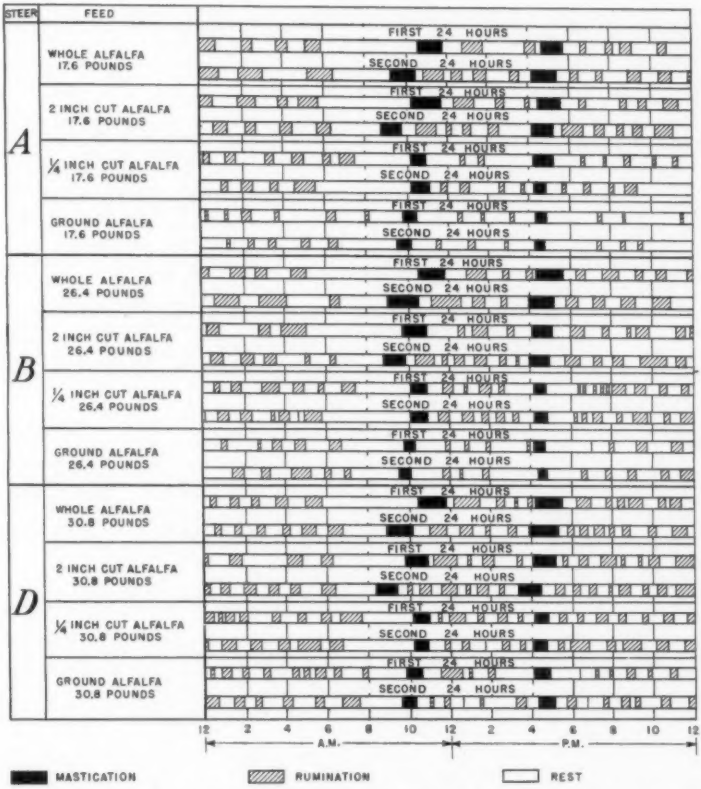

FIGURE 3.—Distribution of periods of mastication, rumination, and rest of steers over a 48-hour period Age of animals: A, 17 to 19 months; B, 22 to 24 months; and D, 31 to 33 months.

finely cut hay, but these differences were small and could only be regarded as an indication.

The mixed rations (5, 6, and 7) required fewer chews and less time for rumination (table 2) than the rations consisting of hay alone. The differences due to grinding the corn were not very consistent, but when the data from all the steers were averaged no effect was demonstrated. However, the removal of the roughage from the ration reduced the rumination process to a minimum as measured by the time and the number of chews taken.

TABLE 2.—*The effect of various methods of preparation of alfalfa hay and of grinding corn on rumination by steers*

[Data are for two 24-hour periods]

Steer	Age	Quantity and preparation of ration	Rumination											
			Chews	Chews per pound	Total time	Time per pound	Portion of day spent ruminating	Boluses	Boluses per pound	Chews per bolus	Periods	Time per period	Time per bolus	Chews per minute
	Months		*Number*	*Number*	*Minutes*	*Minutes*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Minutes*	*Seconds*	*Number*
		ALFALFA HAY												
A	17	8.8 pounds whole alfalfa	19,043	2,164	363	41.3	25.2	350	39.8	54.4	10.5	34.6	62.2	52
		8.8 pounds 2-inch cut alfalfa	19,951	2,267	371	42.2	25.8	365	41.5	54.7	12.0	30.9	61.0	54
	19	8.8 pounds ¼-inch cut alfalfa [1]	15,745	1,789	297	33.8	20.6	312	35.5	50.5	13.5	22.0	57.1	53
		8.8 pounds ground alfalfa [1]	7,536	856	166	18.9	11.5	191	21.7	39.5	12.0	13.8	52.1	45
B	22	13.2 pounds whole alfalfa	22,467	1,702	418	31.7	29.0	488	37.0	46.1	10.5	39.8	51.4	54
		13.2 pounds 2-inch cut alfalfa	22,506	1,705	415	31.4	28.8	543	41.1	41.4	13.5	30.7	45.8	54
	24	13.2 pounds ¼-inch cut alfalfa [1]	22,173	1,680	427	32.3	29.7	533	40.4	41.6	20.0	20.4	48.1	52
		13.2 pounds ground alfalfa [1]	14,867	1,126	299	22.7	20.8	360	27.3	41.3	13.0	23.0	49.8	50
D	31	15.4 pounds whole alfalfa	22,702	1,474	426	27.7	29.6	468	30.3	48.5	15.5	27.5	54.6	53
		15.4 pounds 2-inch cut alfalfa	24,696	1,604	524	34.0	36.4	514	33.4	48.0	17.0	30.8	61.2	47
	33	15.4 pounds ¼-inch cut alfalfa [1]	23,362	1,517	517	33.6	35.9	500	32.5	46.7	17.5	29.5	62.0	45
		15.4 pounds ground alfalfa [1]	15,723	1,021	366	23.8	25.4	365	23.7	43.1	18.0	20.3	60.2	43
		GROUND CORN												
A	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds shelled corn	7,615	544	187	13.4	13.0	282	20.1	27.0	12.5	15.0	39.8	41
	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds ground corn	3,956	283	110	7.9	7.6	125	8.9	31.6	10.0	11.0	52.8	36
B	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	4,527	252	105	5.8	7.3	130	7.2	34.8	8.0	13.1	48.5	43
	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	7,228	402	155	8.6	10.8	181	10.1	40.0	9.0	17.2	51.4	47
	36	2 pounds protein supplement, 14 pounds shelled corn	2,923	183	80	5.0	5.5	85	5.3	34.4	8.0	10.0	56.5	37
D	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds shelled corn	8,673	394	194	8.8	13.5	200	9.1	43.4	14.0	13.9	58.2	45
	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds ground corn	9,817	446	213	9.7	14.8	217	9.9	45.2	14.5	14.7	58.9	46
	42	2 pounds protein supplement, 18 pounds shelled corn	4,066	203	96	4.8	6.7	112	5.6	36.3	8.5	11.3	51.4	42
E	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	10,389	577	246	13.7	17.1	250	13.9	41.6	11.0	22.4	59.0	42
	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	10,126	563	237	13.2	16.5	293	16.3	34.6	14.0	16.9	48.5	43
	28	2 pounds protein supplement, 14 pounds shelled corn	2,120	133	65	4.0	4.5	63	4.0	33.7	5.5	11.8	61.9	33

[1] Several regurgitations before bolus was swallowed.

The total number of boluses regurgitated, the number of chews, and the time spent on each bolus were practically the same when the rations consisted of cut hays or whole hay, but when ground hay or the mixed rations were fed, these indices were greatly reduced, with the exception that the time spent on each bolus was fairly constant on all the rations. Feeding a ration devoid of roughage resulted in a minimum number of boluses being regurgitated and a slight reduction in the number of chews per bolus as compared to the other mixed rations. A peculiar observation was made when the animals were

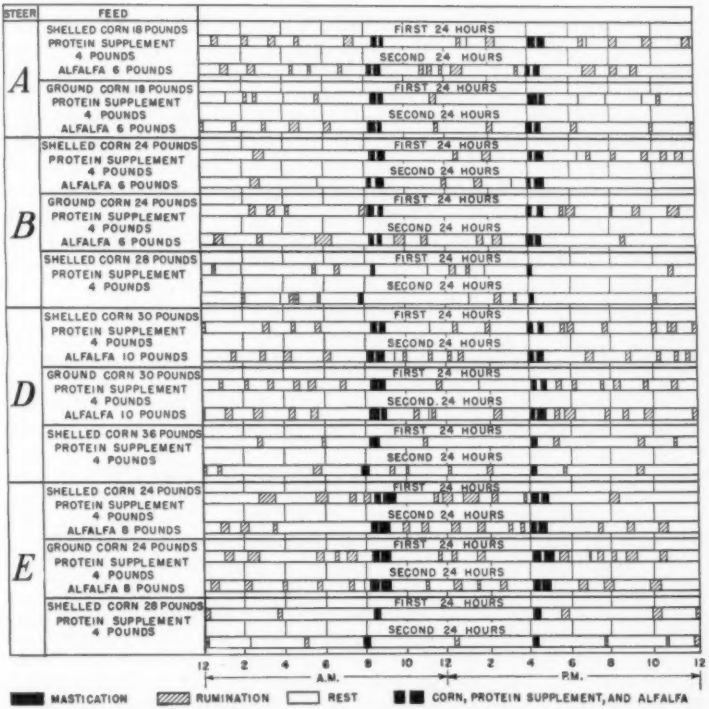

FIGURE 4.—Distribution of periods of mastication, rumination, and rest, and of feedings of steers over a 48-hour period. Age of animals: A, 28 months; B, 33; D, 42; and E, 28 months.

fed the finely ground hay and the mixed rations. Two or three, sometimes as many as six, boluses were regurgitated and remasticated before any of the material in the oral cavity was swallowed.

The rumination process was divided into a number of periods scattered throughout the 24 hours (figs. 3 and 4). The processing of both roughage and concentrates had no apparent effect on the number or distribution of these periods, but it is obvious from the graphs that the length of these periods was reduced materially when ground hay or the mixed rations were fed.

The rate of chewing was practically constant for the whole and cut hays and showed only a slight reduction on the ground alfalfa. However, the rate on the mixed rations appeared to be slower than whe..

roughage alone was fed, and this was further reduced by the entire removal of the roughage from the ration.

DISCUSSION

The object of the mastication of feed is apparently twofold—the comminution of the feed particles and the incorporation of the saliva in the feed. These two processes occur simultaneously and which of them is predominant depends upon several factors. One of the more important of these factors is the physical condition of the feed. In every case except that of Steer A the data show a reduction in the number of chews per pound of feed required for the mastication of alfalfa as the particles became smaller. An average of approximately twice as many chews were made on the whole alfalfa as on the ground alfalfa. This would naturally be expected since comminution of the feed must necessarily have been more important in preparing the whole hay for deglutition than the ground hay. It was actually observed when whole hay was fed that prehension was much slower and the chewing more vigorous and deliberate than when the processed hays were eaten. An average of approximately four times as many chews were made in masticating a pound of whole alfalfa as were necessary for a pound of whole shelled corn and protein supplement, while the number of chews required for ground corn was approximately 25 percent greater than for shelled corn.

All of the observed results may be explained on the basis of the primary object of the mastication of the various rations. When the comminution process was the primary object, as in the case of the whole hay, the maximum number of chews per unit of feed resulted. The insalivation process, taking place simultaneously, was not a limiting factor in the time or number of chews required. As the hay was processed to reduce the size of particles, the comminution process required less time and energy, and therefore the time and the number of chews were reduced. This was true within certain limits. When the hay was finely ground the insalivation process had attained predominance, and the time and energy required were apparently dependent upon this factor.

When shelled corn was fed, prehension was extremely rapid and the corn was swallowed as soon as it was sufficiently covered with saliva to allow easy deglutition. Apparently the reduction in the size of particles was not a factor of major importance in the mastication of this material. This was also true in the case of ground corn, and it appears as though the increased number of chews can only be explained on the basis of the difficulty of insalivation. Shelled corn kernels, protected by their outer coating high in fiber, are not capable of absorbing as much saliva as the soft, starchy endosperm which is exposed by the ground corn. Therefore, it is apparent that it will be more difficult to prepare the ground corn for deglutition, and this explains the increased number of chews and length of time required.

The age of the animal would seem to affect mastication. The younger animals took more chews per unit weight of feed than the older steers.

Other factors which probably affect the mastication process are metabolic rate, general physical condition, and appetite. It is always difficult to measure such factors and evaluate their effects, but some ...ication of their collective effect exists in the data presented. When

the animals were on the higher plane of nutrition of the mixed rations they were in better general physical condition, gained in body weight, and had much keener appetities than when the ration consisted of alfalfa hay alone. The animals spent less time and made fewer chews in the mastication of the mixed ration in spite of the fact that they actually consumed more total feed. Just how much of this effect can be attributed to the factors mentioned is difficult to say, but it seems plausible at least to assume that they played some part in the change.

The most important factor in the amount of rumination observed in the steers was the quantity of roughage in the ration. When rations composed of corn, protein, and hay were fed the number of chews during rumination was approximately 60 percent fewer than when alfalfa was fed alone. This decrease occurred in spite of the fact that the mixed rations contained more pounds of feed than were fed as alfalfa alone. The removal of all roughage from the ration reduced the amount of rumination to a minimum, which was approximately 45 to 50 percent of that occurring on the mixed ration and approximately 15 percent of that on the rations of whole or cut hay. When the ration contained no roughage rumination was slow and listless and had the appearance of being done from an inherent impulse rather than from any desire or necessity. No ill effects were noted on the general health or appetite of the animals when the ration consisted of corn and protein supplement for a period of 2 weeks.

Cutting hay had no effect on the amount of rumination. Since the comminution of the feed particles is usually considered a major object of rumination, it would appear that the differences in the particle size had been minimized by the differences in the amounts of mastication already noted on the different hays. Another possibility is the reduction of the particles in the rumen due to such processes as maceration caused by the motility of the rumen walls, bacterial fermentation, or chemical reactions, which undoubtedly occur continuously. The feeding of ground hay resulted in a 40-percent reduction in rumination. It appeared from observation that reinsalivation was the predominant factor since the chewing was not vigorous and several boluses were regurgitated before deglutition occurred.

Grinding shelled corn had no apparent effect on the amount of rumination.

SUMMARY AND CONCLUSIONS

From the results obtained, it would appear that cutting hay for steers might conserve some metabolizable energy because of a reduction in the energy required for mastication, but no economy was effected because of a reduction in rumination.

Ground hay required less work for both mastication and rumination.

No economy of metabolizable energy was apparent from a decrease in rumination when shelled corn was ground, and grinding actually increased the work required for mastication.

An important factor in the amount of mastication and rumination required on the rations studied was the proportion of grain and roughage in the ration.

Mastication required from 1.4 to 11.0 percent and rumination required from 4.5 to 36.4 percent of the daily time, depending on the ration.

RELATION OF WATER-SOAKED TISSUES TO INFECTION BY BACTERIUM ANGULATUM AND BACT. TABACUM AND OTHER ORGANISMS [1]

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INTRODUCTION

The epidemiology of certain leaf spot diseases of tobacco and other plants is still the subject of much speculation, even though distinct advances in the knowledge of the subject have been made in recent years. Blackfire or angular leaf spot of tobacco, caused by *Bacterium angulatum* Fromme and Murray, is, for example, a widespread and often a serious disease of this crop. Nevertheless, artificial inoculation with this pathogen under ordinarily favorable conditions of environment for infection with many parasites either fails to produce any symptoms, or, at most, produces only small incipient lesions not comparable to the severe necrosis which often develops under field conditions. This situation remained obscure until recently, when it was shown by Clayton (*3*)[2] that the highly necrotic or "epidemic" form of wildfire (*Bact. tabacum* Wolf and Foster) and blackfire is dependent upon the tissues becoming water-soaked by rain followed by the infection of these areas by the pathogen. The water-soaking explanation of infection, as contrasted with the older conception of leaf wounding by rain as a predisposing factor, marked a distinct step in the understanding of certain diseases of tobacco, particularly the blackfire disease. The experimental method in the present investigation differs from that used by Clayton in that water soaking was induced by internal rather than external water pressure.

The demonstration of the relation of water-soaked tissues to infection by organisms and development of disease now presents new problems on the nature of parasitism and of predisposition and susceptibility of plants to disease. In a practical sense, it also raises questions as to the reliance that can be placed on certain types of control measures such as sanitation and eradication. It will be shown in this paper, for example, that water soaking permits infection of normally immune plant species with various bacterial pathogens, and to some degree with bacteria which are not normally pathogenic. The determination of the host range of an organism, and even the definition of parasitism, may become difficult under such circumstances.

METHODS AND MATERIALS

The chief modification of method used in the present studies beyond those commonly employed or previously described consisted essentially of water soaking the plant tissues by means of the application of a high water pressure to the root system or the cut stem end of plants. The principle involved was perhaps first used by De Bary, and has been

[1] Received for publication May 24, 1937; issued November 1937. Cooperative investigations of the Wisconsin Agricultural, Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to Literature Cited, p. 618.

frequently employed since in physiological investigations. The apparatus as used in this laboratory was briefly described in 1924 in connection with plant virus studies (*6*). This equipment consisted essentially of a brass container connection to the pipe line of an ordinary water supply under pressure, together with the necessary valves and drain. This container is fitted with a neck to hold a no. 4 rubber stopper, which is securely held in place by means of a packing box similar to that used around valve stems. Rubber stoppers with holes of different sizes were made to hold plant stems of different sizes. These stoppers may be split down one side, so as to slip readily around the stem of the plants. A split brass washer on the top of the stopper permits the packing-box nut to be screwed down sufficiently by hand without displacement of the stopper. The contact around the plant stem may be quickly and almost perfectly made in this manner and will stand 100 pounds of water pressure without leaks (fig. 1).

The soil was washed off the roots before the root system was placed under pressure. If desired, the root system need not be used, the cut end of the stems only being placed under pressure. Water soaking in some plants starts very quickly, and may cover 50 to 100 percent of the leaf area in 15 minutes. With other species or with individual plants 30 to 60 minutes of exposure may be required to secure a limited amount of water soaking. After the plants were water-soaked to the desired degree, they were atomized with a water suspension of the organism to be tested, then removed from the apparatus and the roots or stem ends placed in a flask of water. The plants were then placed in a chamber with a moisture-saturated atmosphere, where the water-soaked conditions could often be maintained for several days if desired. Evident symptoms usually developed after 18 to 48 hours in the moist chamber, but in cases of uncertain infection or none the plants were left in the moist chamber for as long as 4 days.

Tomatoes (*Lycopersicum esculentum* Mill.) were frequently used as the test plant for parasitism because of the ease with which they were handled and water-soaked, but tobacco was used regularly, particularly in connection with the symptom studies on the blackfire and wildfire organisms. Aside from species of Solanaceae, the other plants used were usually chosen at random from species available at the time in the greenhouse or garden.

The organisms used in the experiments were secured from authoritative sources, largely from workers actively engaged in studies of the organism in the Departments of Plant Pathology or Agricultural Bacteriology at the University of Wisconsin.[3]

EXPERIMENTAL RESULTS

The infection experiments have been conducted for the most part without any special consideration to tobacco (*Nicotiana tabacum* L.) as the typical host to *Bacterium angulatum*. It may be recalled that *Bact. tabacum*, the causal organism of wildfire, was reported as possessing a wide host range (*8*) but that according to Clayton (*2*) only species of *Nicotiana* should be regarded as true hosts. Since the close relationship of *Bact. tabacum* and *Bact. angulatum* has come to be accepted

[3] Thanks are due to several associates for furnishing these cultures, and especially to A. J. Braun, who maintained suitable cultures of *Bacterium angulatum* and *Bact. tabacum* for these studies.

FIGURE 1.—The equipment used for water soaking plant tissues by means of a high water pressure on the root system or cut stems of plants. The packing-box nut holding the split rubber stopper is shown at the base of the stem.

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(*1*, *9*), and the relation of water soaking to infection has been established, the host range of one or both of these organisms will no doubt need reconsideration. If we should limit the true hosts to those only on which infection may be secured artificially by ordinary spraying inoculation, together with those which are found to be infected under natural conditions, we should even then possibly need to consider the true hosts of *Bact. angulatum* and *Bact. tabacum* as extending beyond the genus *Nicotiana*. Manifestly, the influence of both the internal and external water relations of the host may be considered in host-range determinations.

INFECTION WITH BACTERIUM ANGULATUM WITHOUT WATER SOAKING

Some of the conditions aside from external environment which favor infection of tobacco with *Bacterium angulatum* have been frequently suggested, among these being the nutritional balance and height of topping of the crop (*4*), but these factors according to Clayton are not clearly separable from the modified susceptibility of the plants to water soaking (*3*).

The writer's experiments, first carried on with *Nicotiana tabacum* (var. Wisconsin Havana Seed) under greenhouse conditions and without water-soaked tissues, were often made unreliable because of the failure to secure infection in sufficient degree to form a satisfactory basis for interpretation of results. *N. tabacum* is relatively very resistant to *Bacterium angulatum* as compared with *N. glutinosa* L. (fig. 2). For example, a group of 46 inoculations to tobacco under a wide range of temperature (10° to 40° C.) and relative humidity (50 to 100 percent) conditions yielded only 6 plants definitely infected, whereas out of 54 *N. glutinosa* plants under the same conditions, 51 were definitely infected. It is significant that distinct infections on *N. glutinosa* were secured at both the temperature and humidity extremes, and that the atmospheric environment following spray inoculation on the whole appeared to play but a minor role in the amount of infection which developed. Pretreatment of *N. tabacum* for 12 hours or more in a humid atmosphere before inoculation was somewhat more reliable and effective in favoring infection than was the treatment after inoculation.

Repeated spray inoculations in the greenhouse to both *Nicotiana tabacum* and *N. glutinosa* on 27 soils (including soil from all tobacco-growing sections of the United States) differing widely in physical structure and chemical fertility yielded no results which could be definitely correlated with soil type, soil fertility, or rate and vigor of growth of the host. In 10 trials performed at different times, however, it was fairly evident that certain 2 or 3 individual soils regularly yielded plants considerably more susceptible to infection than did other soils, the reason for which could not be determined. Still other modifications, such as inoculation by wiping *Bacterium angulatum* over the leaf surface with cheesecloth so as to break the trichomes, failed to yield good infection. The result of all experiments of this type in the absence of water soaking was the failure to secure at will the large angular or blotchy necrotic symptoms characteristic of heavy field infection on ordinary tobacco. However, good infection of the small necrotic or incipient type may be fairly easily secured on nearly all species of *Nicotiana* (*N. repanda* is most resistant or immune) and on species of several other genera with *Bact. angulatum* in the absence of

water soaking. The following species in particular often yielded fair infection: Tomato (*Lycopersicum esculentum* Mill.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.) (fig. 3), jimsonweed (*Datura stramonium* L.), apple-of-Peru (*Nicandra physaloides* (L.), Pers.), cucumber (*Cucumis sativus* L.), and pokeweed (*Phytolacca decandra* L.). Other solanaceous species such as black nightshade (*S. nigrum* L.), eggplant (*S. melongena* L.), and physalis (*Physalis pubescens* L.) failed to yield even minute amounts of infection under the above experimental conditions. It is of some interest to note

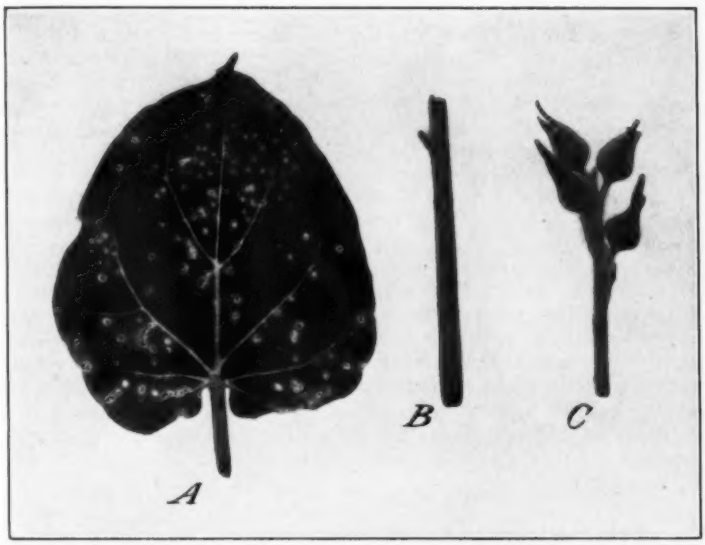

FIGURE 2.—Lesions of *Bacterium angulatum* on leaves (*A*), stems (*B*) and pods (*C*) of *Nicotiana glutinosa*, resulting from spraying inoculation without water soaking. This species is more susceptible than *N. tabacum*, and was frequently used in the trials with ordinary inoculation methods.

that the sterile hybrid of *N. tabacum* (resistant)×*N. glutinosa* (susceptible) is distinctly intermediate in reaction to *Bact. angulatum*.

INFECTION WITH BACTERIUM TABACUM WITHOUT WATER SOAKING

The accumulated experience and observation over a period of years indicates that *Bacterium tabacum* is a more virulent parasite than is *Bact. angulatum*, though admittedly comparative morphological, physiological, and serological studies of the two organisms indicate their close relationship (*1*). The toxin-producing ability of *Bact. tabacum* is, of course, a major difference, and Clayton (*3*) has come to the conclusion from his water-soaking experiments that this is the chief difference between the two organisms as far as infection and pathogenicity are concerned.

Observations in the seedbed and in the field have led the writer to conclude that water soaking is by no means as essential for infection and severe expression of disease with *Bacterium tabacum* as with *Bact. angulatum*. Inoculation of tobacco in the greenhouse by mild spray-

ing has also regularly shown greater virulence in *Bact. tabacum* as measured by the number of infections which develop and the size of the necrotic area. In seeking further experimental proof of this difference, tobacco leaves were inoculated by gently wiping bacterial suspensions from cultures over the leaf surface with cheesecloth, thus breaking the leaf hairs and perhaps occasionally the cuticle. When inoculations are made in this manner *Bact. tabacum* yields heavy infection both as regards number of infections and subsequent necrosis, whereas *Bact. angulatum* yields little or none. Since it might be argued that the wildfire toxin from the culture, and not the bacteria, is responsible for the resultant symptoms, the bacteria were centrifuged out of the suspension in three changes of water, thereby removing the toxin. Wiping inoculation with the bacteria alone, evidently free from perceptible amounts of toxin, yielded almost equally numerous though slower infections (fig. 4, *A*). On the other hand, when the bacteria were removed by heat (the toxin according to Clayton (*2*) being thermostable) and the same method of inoculation employed, there were no symptoms of disease.

These results seem to indicate that, given a favorable external environment of reasonable duration, especially as regards moisture, *Bacterium tabacum* is quite able to enter the cells through wounds, or to enter the stomata and produce lesions of considerable size; whereas *Bact. angulatum* may fail completely to infect under like conditions. Furthermore, this greater virulence does not seem necessarily to be connected with the toxin-producing property of *Bact. tabacum*. It is by no means contended, however, that water soaking does not greatly facilitate infection and the rate of progress of *Bact. tabacum* in the tissues, resulting in extensive necrotic areas, though it seems clear from later experiments that the toxin itself is not of any particular advantage in causing such necroses of the tissues.

WATER SOAKING WITH ARTIFICIAL INTERNAL WATER PRESSURE

The physiology of water soaking of the intercellular spaces of plant tissues by water pressure is not sufficiently understood as yet to warrant extensive discussion. Some species water-soak much more readily than others, e. g., tomatoes much more readily than tobacco. Great differences in individual plants of one variety grown under like conditions may exist, and this difference seems to bear comparatively little relation to the vigor of the plant. A very stunted yellow and red slow-growing tomato, for example, may water-soak quite as easily as a larger, vigorous, rapidly growing plant.

Tomatoes are the most convenient and most susceptible plants with which the writer has worked in infection trials on internally water-soaked tissues. For that reason they have invariably been used as control plants when other species were inoculated to verify both the pathogenicity of the culture used and the favorableness of the subsequent environment under which the plants were placed. Control tomato plants not water-soaked but inoculated were also invariably used in each separate trial.

The greatest interest in connection with *Bacterium angulatum* naturally centers around ordinary tobacco as the host. Comparatively young plants with 8 to 10 leaves but with somewhat elongated internodes (which may be induced by crowding of plants on the bench)

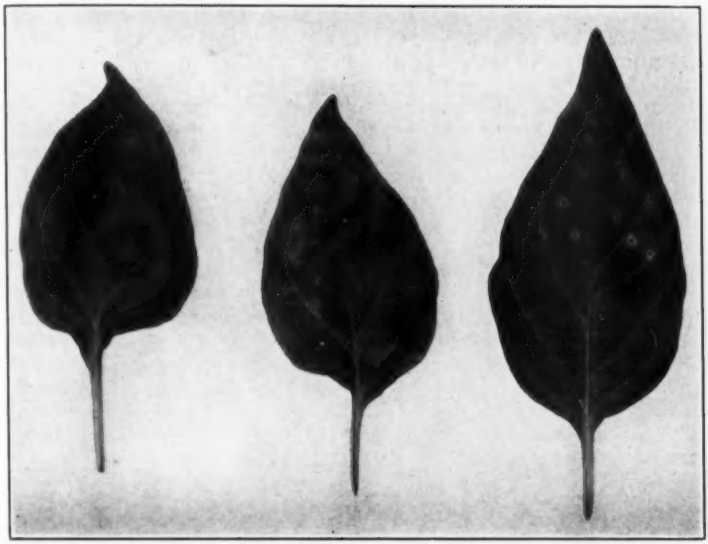

FIGURE 3.—Lesions of *Bacterium angulatum* on pepper secured by ordinary inoculation methods. Many other slightly susceptible hosts outside the Nicotianas have been demonstrated by the same method.

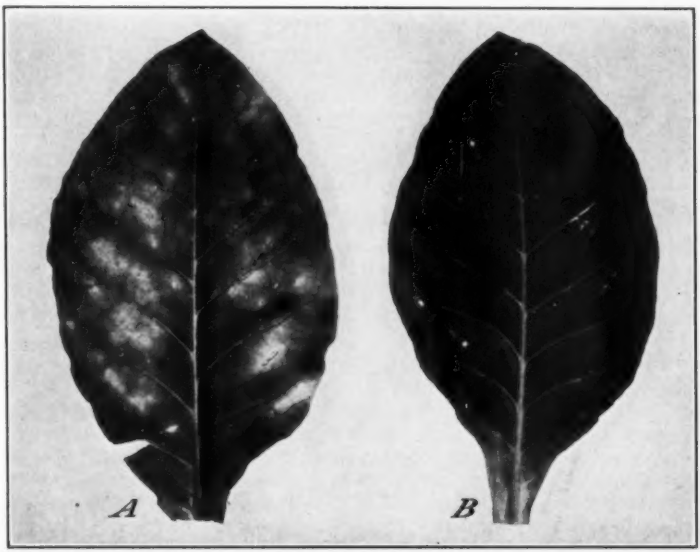

FIGURE 4.—Inoculation of tobacco by the leaf-wiping method, using *Bacterium tabacum* centrifuged free from toxin (*A*) and *Bact. angulatum* (*B*). The infective power of the former in the absence of water soaking is illustrated by the chlorotic areas resulting from numerous young infections. Spots on *B* are due to mechanical injury from wiping.

are most convenient for the equipment used. Twenty to forty minutes of water pressure was often required to water-soak 50 percent or more of the leaf area. The upper leaves usually water-soak more quickly than the older basal leaves, and the intercellular spaces nearest the midrib commonly fill first. However, the soaking is by no means uniform in the tobacco leaves, and the result is usually a wide variety of angles and patterns of varying sizes scattered over the leaf surface, which when allowed to proceed to certain degrees often bears a marked resemblance to the shape and distribution of angular leaf spot disease as it often occurs under field conditions (fig. 5).

If such water-soaked plants are taken out of the apparatus without inoculation and the roots or cut end of the stems placed in water at atmospheric pressure, transpiration at the lower humidities will rapidly remove the excess water in the leaves, but in a saturated atmosphere the water-soaked condition may remain up to 48 hours or more. The uninoculated recovered plants, even though kept under water pressure for 12 hours, show no sign of any physical internal or external injury to the tissues. The freedom from injury may be convincingly demonstrated by spraying water-soaked leaves of an F_1 hybrid (*Nicotiana tabacum* × *N. glutinosa*) with the virus of ordinary tobacco mosaic. If lesions of microscopic size are present, this host will develop marked necrotic lesions of virus infection. This absence of injury to the leaf surface by the internal water-pressure method has some advantages over the externally applied sprays from the point of view of illustrating the basic facts of infection.

The plants were sprayed once or twice with a DeVilbiss atomizer while under pressure, then set into milk bottles or Erlenmeyer flasks in water sufficient to cover the root system or stem end and placed in a saturated humidity chamber at about 25° C. Exposure in this chamber for as short a duration as 3 hours was sufficient to yield subsequent infection with some organisms on the tomato, but ordinarily the plants were left in the chamber until they showed signs of infection, which incubation usually required from 24 to 72 hours.

INFECTION WITH BACTERIUM ANGULATUM IN WATER-SOAKED TISSUES

When tobacco leaves water-soaked by internal pressure are inoculated with *Bacterium angulatum* over the entire surface, the necrotic lesions resulting often correspond closely to the water-soaked areas, and are usually of the irregular and angular type, resembling natural field infection as it often occurs (fig. 6). The blackfire lesions on leaves that are water-soaked by spraying the leaf surface (externally applied water pressure) more rarely show the sharply angular lesions, indicating the delimiting effect of the leaf veins on the lesion. No significance can as yet, however, be attached to this observation. Infections secured by the internal water-pressure method demonstrate in a convincing manner that the organisms enter through the stomata, in the absence of such cuticular wounding as may result from external sprays or storms. Clayton (*3*) suggests that the bacteria are shot directly into the stomata by the force of sprays or rain, and this is borne out in part by the fact that the careful dropping of water suspensions of the organism on the water-soaked area of the leaf is not as likely to yield infection as is light spraying.

FIGURE 5.—A tobacco leaf from a plant water-soaked by the water-pressure method. The angular and speckled character of the lighter colored water-soaked areas is shown.

FIGURE 6.—A leaf of tobacco sprayed with *Bacterium angulatum* following water soaking by the water pressure method. The necrotic spots correspond roughly to the water-soaked areas and resemble the "epidemic" type of blackfire.

Bacterium angulatum, finding the water-filled stomatal chambers and intercellular spaces suitable for growth, multiplies rapidly and soon causes the cells to collapse, thus forming large necrotic areas (fig. 7). This action of the bacteria results in the epidemic or field

FIGURE 7.—Heavy water soaking of tobacco followed by spraying with *Bacterium angulatum* and continued duration of high humidity results in almost complete collapse of the leaves.

type of symptom as contrasted with the small or incipient type of lesion secured from inoculations without prior water soaking. The incipient lesions may, however, also arise from the pathogen's gaining a foothold in a water-soaked area of very small size. However, as Clayton (*3*) points out, for such lesions to develop, the water soaking

must be of sufficient duration to permit the organism to gain a foothold. The writer has found considerable variation in the time required for infection to occur in different host species as well as in individual plants of the same variety.

The greatly increased susceptibility of tobacco to *Bacterium angulatum* by the water-soaking method led the writer to try this organism on other hosts similarly treated. The results were most striking and unusual in many respects. The tomato plant, for example, proved much more susceptible than tobacco to the blackfire organism, and the leaves often collapsed completely in 24 to 48 hours, along with parts of the leaf petioles and younger portions of the stem (figs. 8, 9). Other

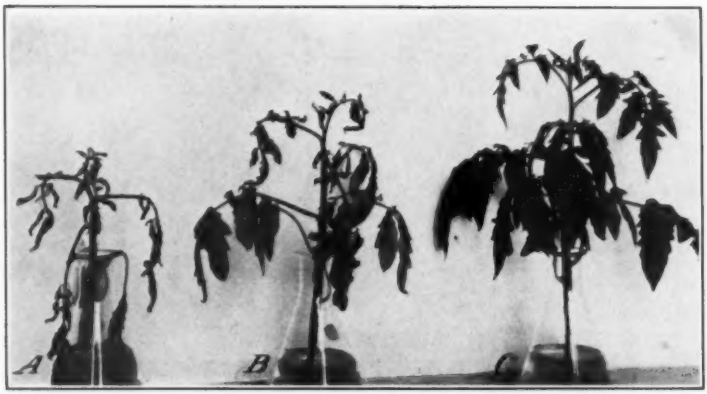

FIGURE 8.—*Bacterium angulatum* on two water-soaked tomatoes (*A*, *B*); control plant (*C*) water-soaked but not inoculated.

solanaceous plants tried, such as potato, eggplant, and datura, were not so markedly susceptible. On the other hand, when still other plants selected at random were used, it became clear that infection with *Bact. angulatum* by this method was not limited to genera or families but spread into a wide variety of unrelated families (figs. 10, 11).

The following species in particular were strikingly infected, some almost as severely as the tomato: Rose (*Rosa* sp.), poinsettia (*Euphorbia pulcherrima* Willd.), locust (*Robinia pseudo-acacia* L.), golden flax (*Linum flavum*), honeysuckle (*Lonicera morrowi* Gray), apple (*Malus sylvestris* Mill.), garden pea (*Pisum sativum* L.), marigold (*Tagetes patula* L.), geranium (*Geranium* sp.), hemp (*Cannabis sativa* L.), bean (*Phaseolus vulgaris* L.), ragweed (*Amaranthus retroflexus* L.), English ivy (*Hedera* sp.), alfalfa (*Medicago sativa* L.), clover (*Trifolium pratense* L.). Species on which infection was not secured with *Bacterium angulatum* included barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), cabbage (*Brassica oleracea* L.), stock (*Matthiola incana* R. Br.), buckwheat (*Fagopyrum esculentum* Moench), snapdragon (*Antirrhinum majus* L.), cactus (*Zygocactus truncatus*), lemon (*Citrus* sp.). Over one-half of the species selected at random from the garden and greenhouse were readily infected with *Bact. angulatum* when inoculations were made to water-soaked tissue. Inoculations

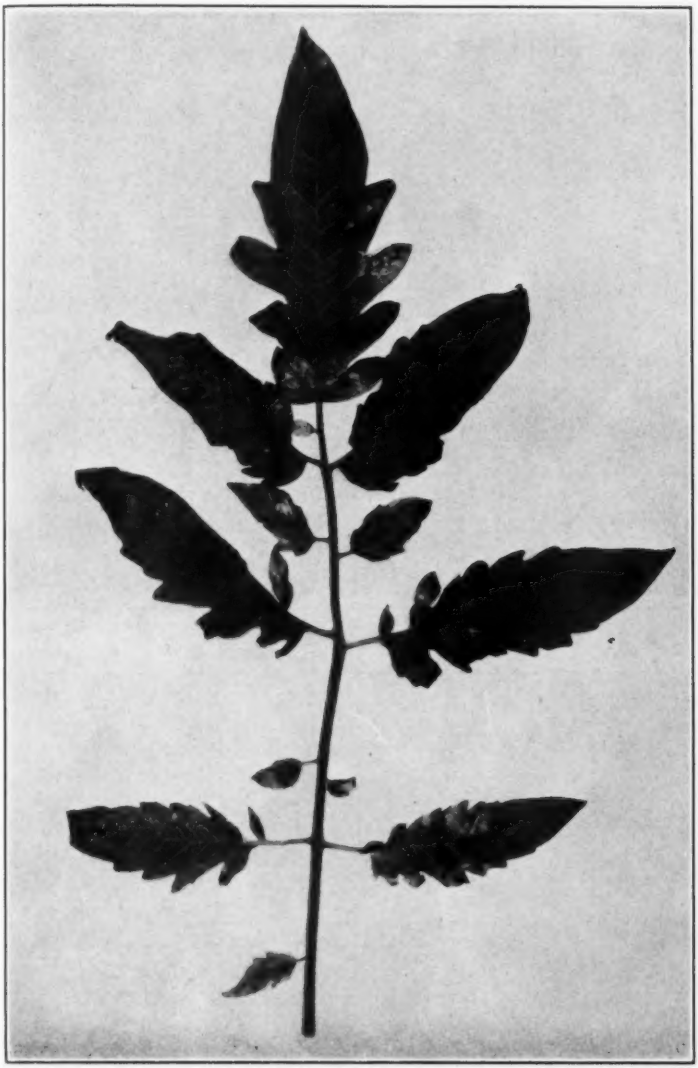

FIGURE 9.—Typical lesions of *Bacterium angulatum* on older water-soaked tomato leaf, which has not wholly collapsed.

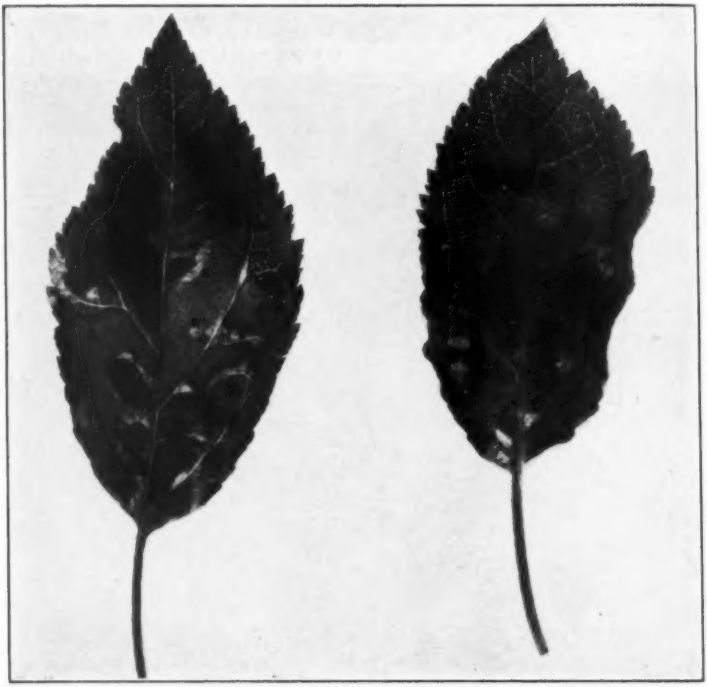

FIGURE 10.—*Bacterium angulatum* on water-soaked leaves of apple. A great variety of plants are equally susceptible after water soaking.

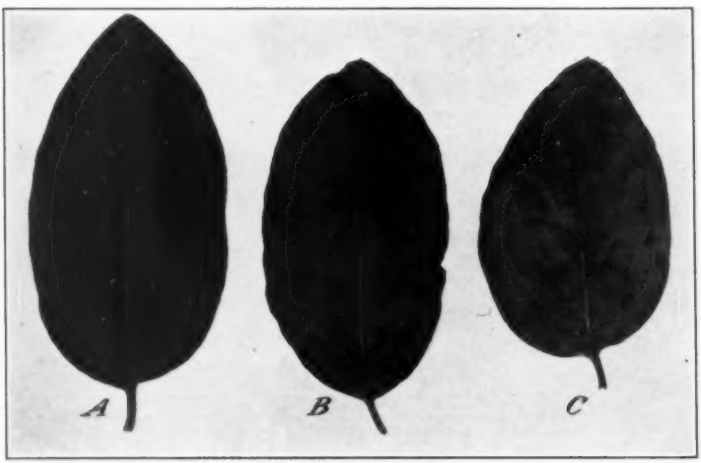

FIGURE 11.—*Bacterium angulatum* on leaves of honeysuckle (*Lonicera morrowi*) (*B*, *C*); control leaf (*A*) water-soaked but not inoculated.

on leaves of these species when not water-soaked yielded no symptoms in this series of trials.

INFECTION WITH BACTERIUM TABACUM IN WATER-SOAKED TISSUES

As is well known, the artificial inoculation of tobacco with *Bacterium tabacum* on tissues that are not water-soaked yields results which are distinct from those secured with *Bact. angulatum*. Under practically all expressions of the two diseases resulting from natural field infection, it is also possible to recognize wildfire because at least some halos are invariably present, and the angular and frequently black lesions of blackfire are characteristic. However, symptoms on tobacco from the two organisms on tissues artificially water-soaked by the internal pressure method are much less distinct and it becomes more difficult and sometimes impossible to separate them on the basis of symptoms.

When tomatoes water-soaked by the internal-pressure method are inoculated with *Bacterium tabacum*, the symptoms are identical with those secured with *Bact. angulatum*. The leaf tissue collapses, turns black, and no typical halos are produced, although should the leaf tissue fail to collapse, some general yellowing of the water-soaked area may be visible. Presumably the cells are killed before the toxin has sufficient time to form and act on the surrounding tissue, and the host or conditions are not sufficiently favorable to support the continued development of the organism and its toxin at the margins of the infected area. The advantage, if any, that the toxin furnishes to the parasitism of *Bact. tabacum* appears to be entirely absent in water-soaked tissues. The only difference that the writer has noted between the two organisms on tomato is the greater virulence of *Bact. tabacum* as indicated by the shorter duration of the water soaking required for the necrotic action to take place. Attenuated (1) strains of wildfire showing no toxin production are quite as virulent as are the normal strains.

Inoculations with *Bacterium tabacum* were made on water-soaked leaves of the same species used in the *Bact. angulatum* infection trials described above. Results almost identical in symptom expression with those found in the *Bact. angulatum* tests were secured in all instances, suggesting again the great similarity and close relationship of these two organisms.

INFECTION WITH OTHER ORGANISMS IN WATER-SOAKED TISSUES

The results with the blackfire and wildfire bacteria on water-soaked tissues can hardly be subject to sound interpretation without giving some consideration to the behavior of other plant parasites, or even saprophytes, under similar circumstances. The field of investigation at once becomes too extensive to be adequately surveyed in a preliminary paper, but a limited number of trials has shown that *Bacterium angulatum* and *Bact. tabacum* are not entirely unique in the above-described respects.

The results with some of the other organisms used have not been so uniformly consistent as those with *Bacterium tabacum* and *Bact. angulatum*, perhaps for the reason that the tomato is not a favorable host plant. Some probability of contamination of inoculated test plants by other organisms, where the same incubation chamber is used simultaneously for different organisms, has also been encoun-

FIGURE 12.—A water-soaked tomato leaf inoculated with *Bacterium phaseoli*. The symptoms closely resemble those secured with *Bact. angulatum* and *Bact. tabacum*, but the progress of necrosis is much slower.

tered. Water-soaked uninoculated controls under these conditions have, however, invariably been free from symptoms. Fifteen other bacteria and two fungi have been tested, mostly in a limited way, especially on water-soaked tomatoes. Eight out of nine trials with *Bact. phaseoli* Smith on tomatoes gave definite and distinctive symptoms. The symptoms were somewhat less severe and slower in developing, but were much like those secured with *Bact. angulatum* or *Bact. tabacum* (fig. 12). Infection was also secured with *Bact. phaseoli* on potato, hemp, alfalfa, and marigold, but not on tobacco. *Bacillus carotovorus* Jones collapsed water-soaked tomatoes rapidly with symptoms readily distinguishable from those previously noted. *Bact. tumefaciens* Smith and Townsend, *Bact. punctulans* Bryan, and *Aplanobacter insidiosum* McC. yielded only small areas of blackened

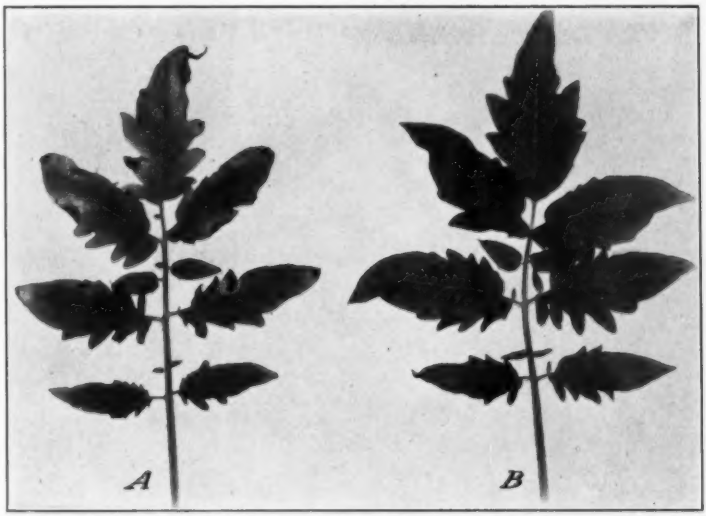

FIGURE 13.—*Macrosporium solani* on water-soaked tomato leaf (*A*) and on leaf without water soaking (*B*).

lesions of some uncertain character; *Bact. stewarti* Smith failed to give any signs of infection on tomato in three trials.

Infection experiments with fungus parasites on water-soaked tissues have been very limited thus far. Infection with *Macrosporium solani* Ell. and Mart. and *Septoria lycopersici* Speg. on tomato was favored by water soaking before inoculation (fig. 13). The latter fungus also evidently attacked potato readily under these conditions. Less conclusive but more surprising were the results sometimes secured with common bacterial organisms not belonging in the category of plant parasites. *Bacillus coli*[4] (Escherich) Migula, *Pseudomonas fluorescens* (Flügge) Migula, *B. radiobactar* Berij. and Van Deld., *B. ruber balticus* L. and N., *B. aerogenes* Kruse, and *Proteus vulgaris* Hauser produced mild necrotic areas on water-soaked tomatoes

[4] *B. coli* has previously been reported as attacking plant tissue. See references in Elliott, Charlotte. Manual of Bacterial plant parasites. Williams and Wilkins Co., 1930.

(fig. 14). Other organisms, including *B. prodigiosus* (Ehren.) Flügge, *B. subtilis* (Ehren.) Cohn, and *Staphylococcus aureus* Rosenbach, failed to produce any symptoms on tomato under similar conditions. In the above group of saprophytes particularly the writer is not prepared to state that true infection was definitely secured, and it should

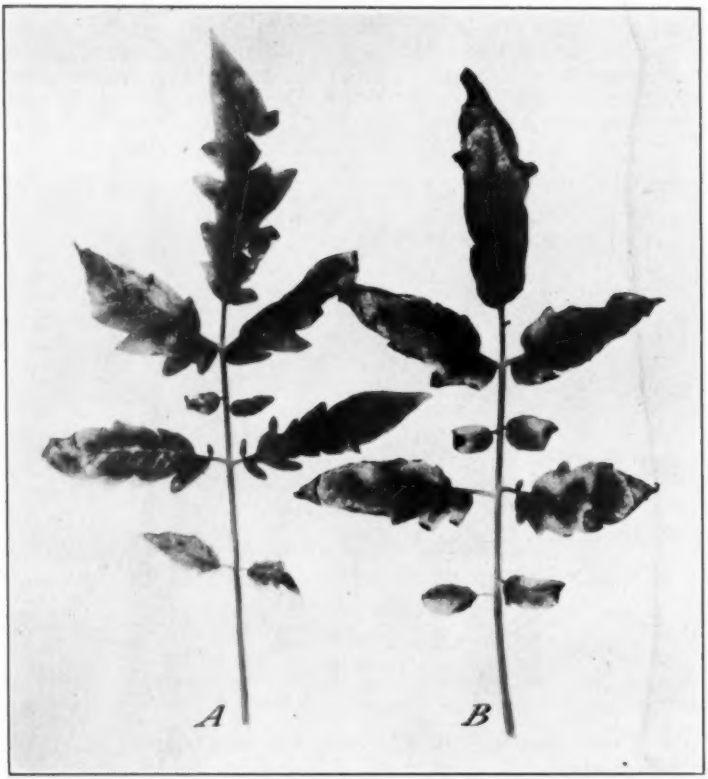

FIGURE 14.—Slow and poorly developed necrosis on water-soaked tomato leaves resulting from inoculation with *Bacillus ruber balticus* (*A*) and *B. aerogenes* (*B*).

be noted here that in no single case in these preliminary trials have Koch's postulates been carried to completion.

DISCUSSION OF RESULTS

It is apparent from the results presented that the infection secured with *Bacterium angulatum* on water-soaked tissues of normally immune species cannot be regarded as a form of saprophytism. The species evidently must be susceptible to attack by certain organisms, since many other species and organisms fail to respond in a similar manner to water soaking.

Since large numbers of organisms have been uniformly applied over the water-soaked areas, we know as yet comparatively little about the rate and distance of the development of the organism through the tissues. It is most likely that the progress from any one point of infection is comparatively localized, and it is obvious that the progress of the organism is limited by the area and the duration of the water soaking. The evidence taken altogether therefore goes to show that *Bacterium angulatum* is a comparatively weak parasite, and, as pointed out earlier by the writer (*5*), the significance of water soaking to infection is likely to be greatest with the less virulent parasites or the relatively more resistant hosts. It is perhaps of little consequence where the boundaries of the parasitism of organisms or the lines of immunity of the hosts are theoretically drawn. This subject resolves itself eventually into a matter of definition. It is significant, however, that these preliminary experiments suggest that a wide variety of normally immune host species may under some circumstances be temporary hosts in nature to *Bact. angulatum*, *Bact. tabacum*, and other organisms, thereby harboring them in such a manner that even the most thorough measures of sanitation and eradication of other known sources of infection may not suffice. Although there is reason to believe that the bacteria in question may persist in such host tissue in a dormant condition for a considerable time (*7*), the proof of this relation remains to be established. It is also possible that many of our so-called nonparasitic leaf spots in nature may be caused by organisms capable of infecting only water-soaked tissue. Isolates from such diseased tissue would naturally fail to give infection upon reinoculation by ordinary methods, and thus lead to erroneous conclusions, as suggested by Clayton (*3*) for the so-called nonparasitic blackfire described by Valleau (*10*).

It is believed that the experimental method of internal water soaking, as described, along with the external method of water soaking as used by Clayton (*3*), may have a wide application in furthering the present understanding of infection and progress of disease in plants. It is not unlikely that in nature a combination of both external and internal water pressure plays a role in predisposition to disease. It follows that more careful observations of water soaking as it occurs in nature, both as a result of beating rain (*3*) and root pressure (*5*), should be more generally made in relation to the epidemiology of plant diseases.

SUMMARY

The intercellular spaces of tobacco and other plant species were water-soaked by applying water pressure to the root system or cut stems, after which they were inoculated with *Bacterium angulatum* and other organisms.

Tobacco is normally very resistant to infection with *Bacterium angulatum*, but when tissues are water soaked, either by external or internal application of water pressure, and this condition is of sufficient duration, the tissues become very susceptible to the organism. No other set of environmental conditions for infection brought about the severe or "epidemic" type of this disease.

It is shown that this situation is not peculiar to tobacco or to *Bacterium angulatum* and *Bact. tabacum*. A wide variety of plant

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species becomes equally susceptible to attack by these same organisms when the tissues are water-soaked. Excellent necrosis was secured on such plants as tomato, alfalfa, bean, pea, hemp, rose, apple, locust, flax, marigold, and poinsettia. These plants are normally immune to infection with these organisms. Other plant species tried were immune in the water-soaked condition.

Other plant parasites, such as *Bacterium phaseoli*, not normally capable of affecting tomato, for example, are capable of causing necrosis when inoculated into water-soaked tissues of this plant. A small amount of necrotic action was also secured on water-soaked tomatoes sprayed with such saprophytic species as *Bacillus coli*.

So far as can be determined, water soaking by the internal water-pressure method does not wound or injure the tissues, showing rather conclusively that the bacteria enter through the stomata, and that cuticular or epidermal wounding caused by rainstorms is not a fundamentally necessary condition for heavy field infection with *Bacterium angulatum* and *Bact. tabacum* as was previously supposed.

Modifications in the present conception of parasitism and immunity as regards definition of the terms are suggested by the results secured with water-soaked tissues. It is also likely that the results may have some practical bearing upon our present understanding of the sources of overwintering of certain plant parasites, and hence may modify the present theories of applying sanitary and eradication measures of disease control.

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EFFECT OF SPECIES OF HOST PLANT ON NITROGEN FIXATION IN MELILOTUS[1]

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INTRODUCTION

Early investigators of symbiotic nitrogen fixation believed that if the organisms invaded the plant and formed nodules there would be adequate fixation of elemental nitrogen, but later work showed this concept to be partly in error. Experiments in the controlled environment of the greenhouse and in the field yielded conclusive evidence that different strains of the bacteria benefit the host plant in varying degrees. By "strain" is meant any pure culture of the root nodule bacteria. In the course of many investigations there was observed a certain relation of bacteria and plant which is called host plant specificity. For example, an organism from vetch may be more beneficial to this plant than an organism from pea, although both strains will form nodules on the vetch. Here two genera are concerned, but in certain genera, notably in *Soja*, species and even varietal differences of response to invasion by a given strain of the proper species of bacteria have been reported. As the older work on strain variation and host plant specificity has been discussed by Fred et al. (*4*),[2] and by Allen and Baldwin (*1*), only a few of the more recent investigations will be reviewed.

Bjälfve (*2*) found differential varietal response among the vetches as to number and position of the nodules and quantity of nitrogen fixed. Virtanen (*8*) states that the most suitable strain of organism for one variety of peas is likewise the best for other varieties. He used only two strains of the pea organism; hence his observation may be true only for these two strains.

Examination of the literature reveals that most of the knowledge concerned with host-plant specificity has resulted from incidental observations in experiments made primarily to study strain variation among the bacteria. Few experiments are recorded in which the object has been to investigate specifically the possible role of the host in conditioning this variation in the organism. Because of the obvious importance and need of such studies, experiments have been conducted at this station for the past 3 years concerned with the specific problem of the influence of the host plant in the symbiosis. In this report are given the results of nitrogen-fixation tests on different species of *Melilotus* when inoculated with pure-culture strains of *Rhizobium meliloti*.

EXPERIMENTAL TECHNIQUE

Ten plants of different species of sweetclover were grown in half-gallon earthenware jars containing 2 kg of nitrogen-poor pit sand into which was inserted a watering tube. The methods for sterilization of seed and sand are given in previous publications (*5, 10*). The plants were watered as needed with sterile, distilled water and once a week

[1] Received for publication May 3, 1937; issued November, 1937. Herman Frasch Foundation in Agricultural Chemistry, Paper no. 138. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin. Technical assistance on this problem was supplied in part by workers employed in the University's Works Progress Administration Natural Science Project.

[2] Reference is made by number (italic) to Literature Cited, p. 629.

with sterile, nitrogen-free Crone's solution (*4*). In order to prevent contamination with foreign strains of the organism, the plants were protected by special chambers. These were made by covering a light wooden framework with a transparent rubber product called "Pliofilm." All jars inoculated with the same strain of bacteria were placed under the same chamber on low wooden trays mounted on rollers. The top of the chamber was hinged to facilitate the addition of water or nutrient solution. The efficacy of the method was demonstrated by the fact that uninoculated controls were thus kept free from nodules, although similar uncovered controls almost invariably became inoculated with stray organisms from the air. The trays bearing the jars were moved daily on the greenhouse bench so that variation arising from position in the greenhouse would be largely eliminated. When the growth of any of the plants was hindered by the light chamber, all were removed and the sand of each pot was covered with a thin layer of sterile cork. The cultures were usually 6 to 8 weeks under the light chambers and 2 to 3 weeks in the open air. At harvest the plants were washed free of sand and dry weight and total nitrogen determinations were made.

The species [3] of *Melilotus* used in these experiments were:

Melilotus alba (Hubam).—An annual-flowering white sweet clover (commercial seed).

Melilotus alba (32–19).—A tall late-flowering biennial from a single plant.

Melilotus suaveolens (F. P. I. 40937).—Redfield Yellow, a biennial developed at South Dakota.

Melilotus officinalis (Y–32–45).—Common yellow sweetclover. Biennial grown at this station for 20 years.

Melilotus officinalis (Y–33–33).—A fine-stemmed selection of yellow sweetclover.

Melilotus dentata (33–49).—Selection of nonbitter sweetclover from a single plant. Original seed was an annual from Peiping, China.

Melilotus dentata (Ac 91–92).—Selection from single plant grown in Wisconsin. Original seed collected in vicinity of Saratov, Union of Soviet Socialist Republics. Biennial.

Melilotus dentata (Ac 92–27).—Selection from single plant grown in Wisconsin. Original seed collected in West Siberia, Union of Soviet Socialist Republics. Biennial.

Melilotus dentata (Ac 96–2).—Selection from single plant grown in Wisconsin. Original seed from Busk, Poland.

The strains of *Rhizobium meliloti* used for artificial inoculation of the plants were:

No. 100—isolated in 1912 from Farmogerm alfalfa culture, a commercial inoculant.

No. 101—isolated in 1915 from United States Department of Agriculture alfalfa culture.

No. 105—isolated from nodule of alfalfa plant at Wisconsin, 1916.

No. 107—isolated from alfalfa culture of H. K. Mulford Co. in 1919.

No. 110—sweetclover cultures obtained from Illinois (Hansen).

No. 111—sweetclover culture from University of Illinois.

No. 113—isolated from sweetclover plant, University of Wisconsin, 1922.

No. 115—isolated from sweetclover plant, University of Wisconsin, 1922.

No. 128—isolated from nodule of bur-clover at Wisconsin in 1928.

No. 129—like 128 but separable by difference in electrophoretic property.

Since isolation the bacteria strains have been cultivated on artificial agar media (*4*), transferred monthly, and stored in an ice box.

EXPERIMENTAL DATA

Experiment 1 (Oct. 15, 1934, to Jan. 4, 1935) was a preliminary experiment in which 10 strains of the organism were used for inoculation of 3 species (2 varieties of 2 of the species) of *Melilotus*. The

[3] The authors express their appreciation to Prof. R. A. Brink, of the Department of Genetics, for the seed used in these experiments.

greenhouse was not provided with artificial illumination, and the plants were not protected with the light chambers. The experiment was started on October 15, 1934, and growth was satisfactory for the first 6 weeks. During the latter part of the experiment the development of the plants was very poor because of insufficient illumination. After 81 days harvest was made; the plants (triplicate jars) of each species (or variety) inoculated with the same same strain of bacteria were combined for analysis. The data are summarized in table 1.

TABLE 1.—*Growth and nitrogen fixation by different species and strains of Melilotus in association with various strains of Rhizobium Meliloti in experiments 1 to 3*

EXPERIMENT 1[1]

Rh. meliloti strain no.	*M. alba* (Hubam)		*M. alba* (32-19)		*M. officinalis* (32-45)		*M. officinalis* (33-33)		*M. dentata* (33-49)		*M. suaveolens*		Total nitrogen
	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	
	Grams	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Milligrams*
100	1.23	34.3	0.94	34.9	1.08	36.0	1.18	36.6	.....	.....	1.15	39.0	180.8
101	1.02	33.2	1.13	41.0	1.02	35.2	1.08	32.3	.....	.....	.47	15.8	157.5
105	.85	28.4	.95	36.9	.62	23.4	.83	28.2	.....	.....	.45	14.1	131.0
107	.98	29.5	.79	30.5	1.12	43.0	.91	32.8	.....	.....	.90	33.2	169.0
110	.89	29.1	.62	24.0	.58	22.8	.78	27.5	.....	.....	.72	26.4	129.8
111	.98	26.8	1.00	40.7	1.32	48.9	1.45	50.0	.....	.....	1.35	47.5	213.9
113	.96	29.8	.95	37.6	.95	31.2	.89	31.7	.....	.....	.82	29.3	159.6
115	.94	30.1	.77	30.6	1.20	45.8	1.13	40.8	.....	.....	.80	29.1	176.4
128	.98	29.8	.86	30.6	1.14	37.6	1.07	34.8	.....	.....	1.12	39.1	171.9
129	.99	27.4	.89	30.6	1.07	37.2	.83	24.4	.....	.....	.85	29.0	148.6

EXPERIMENT 2[2]

100	4.35	81.8	2.92	80.5	3.68	88.9	.....	.....	2.41	54.5	3.20	83.6	389.3
105	3.34	75.0	3.06	74.6	3.15	78.0	.....	.....	.67	12.2	.82	21.8	261.6
110	3.07	70.5	2.93	75.6	3.14	80.0	.....	.....	3.23	68.0	4.06	92.5	386.6
115	3.27	80.3	2.43	65.0	3.38	79.9	.....	.....	3.15	73.0	3.54	90.6	388.8
128	2.88	71.0	2.42	65.4	2.76	80.0	.....	.....	1.97	44.5	3.76	97.5	358.4

EXPERIMENT 3[3]

100	{ 0.64	15.4	.....	.....	0.68	17.6	.....	.....	0.64	17.6	0.61	15.6	} 144.9
	{ .71	16.4	.....	.....	.92	22.0	.....	.....	.72	20.6	.68	19.7	
Average	.....	15.9	.....	.....	.....	19.8	.....	.....	.....	19.1	.....	17.7	
105	{ .48	13.4	.....	.....	.75	13.1	.....	.....	.19	3.2	.15	3.4	} 71.3
	{ .46	13.7	.....	.....	.70	12.6	.....	.....	.30	7.1	.22	4.8	
Average	.....	13.6	.....	.....	.....	12.8	.....	.....	.....	5.2	.....	4.1	
110	{ .98	18.5	.....	.....	.53	8.4	.....	.....	.84	21.6	.57	13.8	} 117.0
	{ .48	9.0	.....	.....	.87	11.3	.....	.....	.80	20.8	.60	13.6	
Average	.....	13.8	.....	.....	.....	9.8	.....	.....	.....	21.2	.....	13.7	
115	{ .79	14.7	.....	.....	.67	19.5	.....	.....	.80	17.9	1.20	13.7	} 146.6
	{ .68	15.5	.....	.....	.67	19.5	.....	.....	.93	25.4	.70	20.4	
Average	.....	15.1	.....	.....	.....	19.5	.....	.....	.....	21.7	.....	17.1	
128	{ .84	18.7	.....	.....	.35	9.4	.....	.....	.79	17.6	.97	23.4	} 123.6
	{ .83	13.7	.....	.....	.29	4.4	.....	.....	.41	11.8	.90	24.6	
Average	.....	16.2	.....	.....	.....	6.9	.....	.....	.....	14.7	.....	24.0	
Total	.....	149.0	.....	.....	.....	137.8	.....	.....	.....	163.6	.....	153.0	

[1] Dry weights per 10 plants; each datum based on analysis of 25 to 30 plants.
[2] Dry weights per 10 plants; each datum based on analysis of 30 plants.
[3] Dry weight per 10 plants; each datum based on analysis of 10 plants. Underlined values=mean of duplicates. Differences necessary for significance: Between means, 4.32 mg; between totals of strains, 17.3 mg. Differences between totals of species not significant (see table 4).

On the basis of the results obtained in the preliminary experiment five strains of the organism were shosen for further study. These were used for inoculation of plants of four species. The test was carried on from April 2 to June 23, 1935 in a greenhouse especially designed for control of environment in nitrogen-fixation studies (*10*), and the light chambers were introduced to prevent contamination. Growth and fixation of nitrogen were most satisfactory, as can be seen from the data in table 1 (experiment 2).

Experiment 3 (Sept. 30 to Dec. 15, 1935) was a replicate of experiment 2 except that only one variety of *Melilotus alba* was included. The work was done in rainy, cloudy weather, and in spite of artificial illumination the growth of the plants and the fixation of nitrogen were poor. However, this poor development was not entirely undesirable since the experiment was made in order to determine whether season and consequently development of plants affected the relationships between the host and invading bacteria.[4] The data are given in table 11. The data in the total columns are discussed in connection with the statistical analysis.

The data of experiment 1 show two types of variation in growth and fixation of nitrogen by *Melilotus* when inoculated with different strains of *Rhizobium meliloti*. With a given species of the host plant the benefit derived, as measured by the quantity of nitrogen fixed, varied with the strain of bacteria used. For example, with *M. officinalis*, strains 105 and 110 were definitely poor; strains 107, 111, and 115 were superior; the remainder were intermediate. With *M. suaveolens* strains 101 and 105 were poor and 100, 111, and 128 were distinctly good. This type of variation is the usual one noted by numerous investigators (*1*, *4*).

There are, however, also differences in nitrogen fixation of the several species of the host plant when inoculated with certain strains of the organism. For example, *Melilotus suaveolens* fixed less nitrogen in association with *Rhizobium meliloti* 101 and *Rh. meliloti* 105 than did any of the other species. These differences did not arise from variation in the growth habits of the various species of plant since with certain strains of bacteria (e. g., strains 100 and 110) no significant difference in the quantity of nitrogen fixed was found among the several species.

Confirmation of the existence of species variation with respect to fixation of nitrogen in *Melilotus* is obtained from the results of experiment 2 (table 1). This experiment was made under conditions ideal for fixation of nitrogen. The results show that with *M. alba* and *M. officinalis*, all the strains employed were apparently of equal benefit, but with *M. dentata* fixation was only fair with strain 128 and distinctly poor with strain 105. As noted in experiment 1, fixation by *M. suaveolens* in association with strain 105 was likewise very much less than with the other strains.

It is of interest to compare the results of experiment 2 with those of experiment 3 (table 1), made under environmental conditions that restricted fixation of nitrogen. In experiment 3 the analysis of the duplicate cultures was kept separate since, in contrast to the first two experiments, certain of the strains gave erratic reponses, a result that

[4] REID, J. J. THE INFECTIVE ABILITY OF RHIZOBIA OF THE SOYBEAN, COWPEA, AND LUPIN CROSS-INOCULATION GROUPS. Unpublished thesis, Ph. D., Univ. Wis. 1936.

has been observed by other investigators. Thus Wilson, Hopkins, and Fred (*11*) report that alfalfa plants inoculated with *Rhizobium meliloti* 101 and grown under cotton plugs (which also restricts uptake of elemental nitrogen) were benefited by the presence of this strain of the organism in only 7 of the 20 replicate cultures. Löhnis (*6*) reports a similar experience with crimson clover grown in open sand cultures when inoculated with *Rh. trifolii* 205. In general, the results of experiment 3 confirm those of the previous experiments in that both strain variation among the organisms and species variation among the host plants with regard to nitrogen fixation are apparent.

Further investigations of species variation in *Melilotus* were undertaken with those species that had exhibited the widest variation in the first three experiments. No more seed of *M. dentata* (33–49) was to be had, but seeds of three other strains of this species became available. These were tested with *M. alba* (32–19) and *M. suaveolens* in a preliminary experiment with *Rhizobium meliloti* strains 100, 105, and 128 as the source of inoculum. The average nitrogen fixed per 10 plants is shown in table 2.

TABLE 2.—*Nitrogen fixation by different species and strains of Melilotus in association with 3 different strains of Rhizobium meliloti in a preliminary experiment* [1]

Species or strain	*Rh. meliloti* 100	*Rh. meliloti* 105	*Rh. melilot* 128
	Milligrams	*Milligrams*	*Milligrams*
M. alba (32–19)	186. 3	111. 0	147. 5
M. suaveolens	121. 0	11. 0	126. 0
M. dentata (91–12)	149. 0	95. 0	131. 0
M. dentata (92–27)	131. 0	35. 8	142. 4
M. dentata (96–2)	111. 5	142. 0	138. 2

[1] Per 10 plants.

The results of this experiment were very similar to those already discussed, with one exception. Of the three strains of the biennial *Melilotus dentata* used, only one, *M. dentata* (92–27), responded to inoculation with *Rhizobium meliloti* 105 as did the annual, *M. dentata* (33–49). Fixation of nitrogen was fair to good with *M. dentata* (91–12) and was good with *M. dentata* (96–2). This result indicates that the importance of the host plant in nitrogen fixation by *Melilotus* is not confined to species differences but may likewise include varietal (or strain) differences.

In order to examine this problem further two additional experiments (nos. 4 and 5) were made. The results of these experiments are given in table 3. Only the nitrogen fixation data are included since these are the essential ones and the dry-weight figures were consistent with these.

The results of the two experiments confirm in every way those of the previous ones; viz, the existence of three types of response in the association of *Rhizobium meliloti* and *Melilotus*.

(1) With *Rh. meliloti* 100 and 110 fixation of nitrogen by all the species of host plants was good to excellent with little evidence of consistent significant differences among the several species. The results were consistent in that agreement between duplicate samples was satisfactory.

(2) With *Rh. meliloti* 105 evidences of the effect of the host plant were quite pronounced. Fixation of nitrogen in association with *M. alba* (32–19) and *M. dentata* (91–12) was fair to good, but fixation with both *M. suaveolens* and *M. dentata* (92–27) was poor. The response was quite definite and the agreement between duplicates was satisfactory.

In experiment 4 (Oct. 23, 1936, to Feb. 2, 1937) three species of *Melilotus* were inoculated with the same five strains of organisms which were used in the earlier experiments. *M. Alba* (32–19) was used as representative of the *M. alba* species since it had shown more indication of variable behavior with the organisms used than had the Hubam variety. Likewise, the two strains of *M. dentata* which had shown the greatest contrast in the preliminary experiment were selected for further study. The experiment was made during the winter months, and in spite of the use of supplemental lighting, fixation of nitrogen was only fair even with the best of the associations.

In experiment 5 (Feb. 16 to May 21, 1937) only *Rhizobium meliloti* 105, 115, and 128 were used, as the results in the previous experiments indicated that there existed little, if any, consistent differences in the response of the species of host plant under investigation when inoculated with strains 100 and 110. The experiment was started in the late winter and the initial growth of the plants was slow. Later in the spring months growth was excellent and fixation was the greatest obtained in any of the experiments, due in part to the longer period of growth allowed the plants.

TABLE 3.—*Nitrogen fixation by different species and strains of melilotus in association with various strains of rhizobium meliloti in experiments 4 and 5* [1]

Rh. meliloti strain no.	*M. alba* (32–19)		*M. suaveolens*		*M. dentata* (91–12)		*M. dentata* (92–27)		Total	
	Experiment 4	Experiment 5	Experiment 4	Experiment 5	Experiment 4	Experiment 5	Experiment 4	Experiment 5	Experiment 4	Experiment 5
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
100	20.9 23.3		24.1 22.0		20.5 21.5		22.1 28.1		181.5	
Average [2]	22.1		23.1		22.0		25.1			
105	15.4 17.4	163.3 213.0	4.1 3.2	12.9 10.3	15.5 18.3	240.5 268.1	5.8 7.4	55.0 40.0	87.1	1,003.1
Average [2]	16.4	188.2	3.7	11.6	16.9	254.3	6.6	47.5		
110	21.3 20.2		23.3 25.6		19.5 20.5		30.2 32.0		192.6	
Average [2]	20.8		24.5		20.0		31.1			
115	23.1 20.9	144.0 128.6	26.9 30.7	250.2 284.0	27.4 31.8	224.2 230.6	10.2 11.0	117.5 215.0	182.0	1,594.1
Average [2]	22.0	136.3	28.8	267.1	29.6	227.4	10.6	166.3		
128	28.2 27.5	238.0 294.1	18.1 27.3	248.1 170.1	22.6 28.6	193.5 209.5	14.1 24.1	227.5 238.6	190.5	1,819.4
Average [2]	27.9	266.1	22.7	209.1	25.6	201.5	19.1	233.1		
Total	218.2	1,181.0	205.3	975.6	228.2	1,366.4	185.0	893.6		

[1] All values in milligrams per 10 plants. Differences necessary for significance: Experiment 4, between means, 4.33 mg.; between totals of strains, 17.3 mg.; between totals of species, 19.3 mg. Experiment 5 between means, 48.7 mg.; between totals of strains, 194.8 mg.; between totals of species, 168.7 mg.

[2] Mean of duplicates.

(3) In association with strains 115 and 128 certain species exhibited another type of variation with respect to fixation of nitrogen. All the species fixed appreciable quantities of nitrogen with these two strains, but in association with *M. suaveolens* and *M. dentata* (92–27) the response was inclined to be erratic, with the result that agreement between the duplicates was poor. This type of response was discussed in connection with experiment 3, and its possible significance will be taken up later. The time element in the development of the differences between duplicates in experiment 5 is of interest. In the early stages of growth both cultures of a given treatment developed slowly with little sign of difference between them. As the days became longer and brighter one of the cultures would respond to the environmental conditions more favorable for the fixation of nitrogen sooner than its duplicate, with the result that differences in fixation of nitrogen soon became apparent between the two. Later both cultures would actively fix nitrogen, but the one in which the onset of fixation was delayed could not overcome the initial advantage of the other so that the agreement between the duplicates was poor. It should be observed that this difference between duplicate cultures in the onset of the fixation process appeared to be definitely associated with only certain species when inoculated with certain strains of the organism.

The conclusions reached by inspection of the data were confirmed by subjecting them to an analysis of variance (*3*). Such an analysis allows separation of the different sources of variation in the results and enables the establishment of the significance of variation arising from differential response of species of host plant with strain of bacteria. Analyses of variance (table 4) were made on the data from experiments 3, 4, and 5 as in these experiments the duplicates were not combined. The values necessary for significant (19:1 odds) differences between the means of the duplicate samples of each experiment (calculated from the error of the experiment) as well as for total nitrogen fixed by the various strains of bacteria or by the various species of host plant are indicated in the footnotes to tables 1 and 3.

TABLE 4.—*Summary of analyses of variance on experiments 3, 4, and 5*

Variance due to—	Experiment 3			Experiment 4			Experiment 5		
	Degrees of freedom	Variance	F [1]	Degrees of freedom	Variance	F [1]	Degrees of freedom	Variance	F [1]
Strains	4	115.3	13.31++	4	253.8	29.6++	2	21,634.2	21.6++
Species	3	10.86	1.26–	3	33.76	3.94+	3	6,301.6	6.3++
Species × strain	12	51.0	5.92++	12	72.2	8.42++	6	13,946.1	13.9++
Error	20	8.63		20	8.58		12	1,000.3	

[1] If F exceeds value for 5-percent point (indicated by +), the odds are at least 19:1 that observed differences do not arise from chance (experimental error). Similarly, if F exceeds 1-percent point (indicated by ++) odds are 99:1. Minus indicates differences could have arisen from chance.

The source of variation of chief interest is the interaction of species and strain (species×strain), for if this is significant the fixation of nitrogen by a given strain of the organism in association with the host plant is not independent of the species of the host. The analyses show that, in spite of considerable experimental error introduced by

reason of the difference in duplicate samples that has been already discussed, the variation due to interaction of strain and species is highly significant in each of the experiments.

Likewise, in the three experiments for which analyses of variance are reported (table 4) there are significant differences due to strain. Consideration of table 1 (experiment 3) and table 3 indicates that this difference arises primarily because strain 105 is distinctly inferior to the others when the results on all the species are combined. It is to be emphasized that this does not mean that strain 105 is inferior on all the species when considered individually as with certain of them; e. g., on *Melilotus alba* (32–19) and *M. dentata* (91–12) strain 105 usually fixed as much nitrogen as did the other strains. Because of the consistently poor fixation by strain 105 in association with *M. suaveolens* and *M. dentata* (33–49) and (92–27), however, the total fixed by strain 105 is significantly lower than the totals fixed by the other strains used.

Similarly, the significance of the species variation arises from distinctly lower totals for *Melilotus suaveolens* and *M. dentata* (92–27), lower totals which originate primarily from the poor fixation when inoculated with strain 105. With other strains of the bacteria, e. g., strain 100, the quantity of nitrogen fixed by either of these two species is not significantly different from the nitrogen fixed by the other species of host plant.

DISCUSSION

From the results of the foregoing experiments it is concluded that the interaction of bacteria and host plant in symbiotic nitrogen fixation by *Melilotus* may be separated into three distinct types of response with respect to nitrogen fixation.

(1) The association of all the species tested with certain of the organisms is consistently of equal effectiveness. The best examples of this type are *Rhizobium meliloti* 100 and 110 in association with the species under investigation. Probably other strains of the bacteria tested only in experiment 1 would also fall in this class with these particular species of host plant. It should be noted, however, that other species (or even varieties) of *Melilotus* may be found in which the association with strains 100 or 110 would be ineffective.

(2) A second type of association is that in which the species of host determines whether or not the strain of bacteria is effective. For example, *Rh. meliloti* 105 is effective in association with *M. alba*, *M. officinalis*, and *M. dentata* (91–12) but rather ineffective with *M. dentata* (33–49) and (92–27) and with *M. suaveolens*. This type is likewise consistent; i. e., the response of a given species to inoculation with the strain is relatively independent of the environmental conditions under which the experiment is made.

(3) In the third type of association observed in these experiments certain of the host plants and strains exhibited a variable response which appeared to be related to the environmental conditions under which the experiment was made, especially length of day and intensity of light. The most consistent examples of this type was the association of *Rh. meliloti* 115 and 128 with *M. suaveolens*, *M. dentata* (33–49) and (92–27). It is of interest that these species of host plant are the ones which are ineffective in association with *Rh. meliloti* 105.

In many cases the variation is apparent between replicates in the same experiment. The cause of this irregularity is even more obscure than the cause of the differences in effectiveness between individual strains, but it is doubtless a reflection of a fundamental change in the delicate equilibrium existing between host and invader which determines whether the relationship becomes helpful or harmful to the plant. In view of the importnce of the carbohydrate-nitrogen relationship of the host in influencing both effectiveness and ineffectiveness (*9*), it appears that this relationship may be concerned in the inconsistencies noted with certain strains and species. This suggestion receives support from the fact that irregularities are more frequently observed when the experiments are carried out under conditions which favor a narrow carbohydrate-nitrogen balance in the, plant and which consequently restrict fixation of elemental nitrogen at least during the early stage of development. It is further suggested that the inconsistencies in the quantity of nitrogen fixed through certain associations of host plant and bacteria may arise from differences in time of infection by the bacteria rather than irregularities in the effectiveness of the association. Unpublished results by Reid[5] have indicated the importance of the carbohydrate-nitrogen relation in determining whether or not a given strain of the bacteria will infect certain of the host plants of the soybean-cowpea cross-inoculation group. Under an unfavorable environment, invasion of individual plants by certain strains of the bacteria may occur at different periods, depending on what appears to be minor factors in the environment, but which bring about changes in the composition of the host plant sufficient to control the time of infection.

The implication of the findings for theoretical aspects of symbiotic nitrogen fixation is the emphasis which they place upon the influence of the host plant in the symbiosis. The assumption that the host plant passively supplies the source of energy and that the actual process of fixation is a function of the bacteria alone appears to be untenable in the light of these results. Research in the field has been largely concerned with efforts to determine the individual roles of the plant and organism, but such an approach may be unwise. Regarding the fixation of nitrogen as a function of the relationship between host and organism acting as a unit rather than as isolated components, might prove to be a more rational point of view for future research.

A corallary of this view of mutual interdependence of bacteria and plant is the necessity of change in the concept of what constitutes "strain variation"—a broader definition which would include the host plant might be advisable. With our present definitions *Rhizobium meliloti* 105 would be classed as "effective" with certain hosts but "ineffective" with others. But if the variation is considered as one involving the relationship between plant and bacteria rather than merely the strain of the organism (or the species of plant) a more unified conception is possible.

According to this concept the difference in strains of the organism is not so much a definite qualitative one capable of classification in terms as "effective" and "ineffective", but rather a quantitative difference in the manner in which the bacteria affect the relationship between themselves and the host. Moreover, this quantitative

[5] Reid, J. J. See footnote 4.

difference is not necessarily constant but may vary in relation to the presence and activities of other factors which likewise affect the relationship. These factors would include the species of the host plant, the carbohydrate-nitrogen relation in the plant, and probably others; such as day length, temperature, and light intensity. Thus the irregularity in the fixation of nitrogen with some cultures, as *Rhizobium meliloti* 128 with *Melilotus dentata* (33–49) and *M. suaveolens*, *Rh. meliloti* 101 with *Medicago sativa* (Grimm) (*12*), and *Rh. trifolii* 205 with *Trifolium incarnatum* (*6*), constitute examples of alteration of the quantitative effect of a strain on the association through the predominance of some other factor—possibly the carbohydrate-nitrogen relation in the plant—which affects the relationship. The objective of future research is to define the as yet unknown factors, to determine their relative significance especially in combination with one another, and finally to devise means for their control.

SUMMARY AND CONCLUSIONS

Four species of sweetclover, *Melilotus alba*, *M. officinalis*, *M. suaveolens*, and *M. dentata*—were tested for ability to fix atmospheric nitrogen in association with different strains of *Rhizobium meliloti*. Six experiments were made during different seasons with resulting differences in extent and rate of nitrogen fixation by the plants.

Three types of response with respect to fixation of nitrogen were observed in these experiments:

(1) The association of certain of the species of host plants and strains of bacteria were consistently effective.

(2) The association of certain strains of bacteria was effective with one species of the host but ineffective with another. The reverse was also observed, i. e., certain species of the host plant were benefited through association with some strains of the organism but not with others.

(3) The association of certain species of plant and certain strains of the organism gave rise to erratic responses between experiments as well as within an experiment. There was some evidence that this type of response may have been influenced by the carbohydrate-nitrogen relationship in the plant.

These results stress the importance of the host plant in determining whether a given strain of bacteria is effective or ineffective in the association. It is proposed that the concept of strain variation in the bacteria may be only one aspect of a broader type of variation which involves the total relationship between organism and plant. The variation in this relationship with respect to nitrogen fixation may be affected by factors other than differences of strain of bacteria, as, for example, species of host plant or physiological balances within the plant. According to this view a given strain of the organism is not "good" or "poor" in an absolute sense, but only relative to the other factors which affect the relationship. Under certain conditions one factor, as, for example, strain of organism, may determine the effectiveness of the relationship, whereas under other conditions some other factor, such as species of host plant, may be the dominating element.

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U. S. GOVERNMENT PRINTING OFFICE: 1937